Division of

Cancer Treatment

1986 Annual Report
Volume I

October 1, 1985-
September 30, 1986

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

National
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ANNUAL REPORT

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

October 1, 1985 through September 30, 1986

The Division of Cancer Treatment (DCT) is the organizational component of the National Cancer Institute (NCI) responsible for the development and evaluation of new therapies for the control and cure of cancer. The program is carried out in intramural laboratories and clinics as well as through grant-, contract-, and cooperative agreement-supported projects throughout the Nation and the world. Major emphasis in the treatment research program is on optimal integrated use of all modalities of therapy, including chemotherapy, surgery, radiation therapy, biologic response modifiers, immunotherapy, and hyperthermia, used both individually and in combination. The Division has five major programs: Developmental Therapeutics Program (DTP), Cancer Therapy Evaluation Program (CTEP), Radiation Research Program (RRP), Clinical Oncology Program (COP), and the Biologic Response Modifiers Program (BRMP). Each of these programs has a separate and distinct responsibility, encompassing research on the development and identification of new agents or methods for the treatment of cancer: their evaluation in preclinical tumor systems; the testing and evaluation of the safety of new agents or methods and preparation of investigational new drug applications (INDs) for submission to the Food and Drug Administration for approval to initiate clinical trials; the conduct of clinical trials at the earliest level (Phase I); the conduct of disease-specific clinical trials (Phase II); the conduct of clinical trials comparing new and standard treatments (Phase III); and the evaluation of combined modalities of therapy.

For scientific and administrative direction the DCT relies heavily on the advice of the Board of Scientific Counselors, whose membership and affiliations are shown in Table I. The Board represents outstanding extramural scientific talents in the fields of surgical oncology, immunology, radiotherapy, and medical oncology. These represent the areas of clinical science particularly relevant to the research mission of the Division.

The Division Director relies on the Board of Scientific Counselors not only for scientific and administrative advice but also for fiscal direction; for example, all new concepts for contracts or requests for application (RFAs) must meet with Board approval. In addition, Board members serve as chairpersons and members of regular site visits to intramural labs. The Board's counsel is invaluable in ensuring that the best intramural labs receive appropriate support while areas of lower scientific priority are de-emphasized.

Personnel and Organization

The DCT is operationally divided into five major components of treatment program priorities. Each program is headed by an Associate Director who is responsible for the overall direction of the science within that program. A current organizational chart, shown in the following pages, reflects changes that have occurred during the past year. Changes in personnel include the following:

A. Office of the Director (OD)

- No personnel changes

TABLE I. DCT BOARD OF SCIENTIFIC COUNSELORS

Name	Affiliation	Term of Appointment
Samuel A. Wells, Jr., M.D.	Washington University School of Medicine	1982-1986
Dani P. Bolognesi, Ph.D.	Duke University Medical Center	1982-1986
David G. Bragg, M.D.	University of Utah Medical Ctr.	1982-1986
Paul Calabresi, M.D.	Roger Williams General Hospital, Brown University	1982-1986
Max D. Cooper, M.D.	University of Alabama Medical Center	1982-1986
Lawrence H. Einhorn, M.D.	Indiana University Medical Ctr.	1985-1989
Mortimer M. Elkind, Ph.D.	Colorado State University	1982-1986
Karen K. Fu, M.D.	University of California, San Francisco	1983-1987
I. David Goldman, M.D.	Medical College of Virginia	1982-1986
Leon Goodman, Ph.D.	University of Rhode Island	1982-1986
Robert L. Goodman, M.D.	University of Pennsylvania	1984-1987
Susan B. Horowitz, Ph.D.	Albert Einstein College of Medicine	1982-1986
John H. Kersey, M.D.	University of Minnesota Hospitals	1984-1988
Rodrigue Mortel, M.D.	Milton S. Hershey Medical Ctr., Pennsylvania State University	1983-1987
Efraim Racker, M.D.	Cornell University	1982-1986
Alan S. Rosenthal, M.D.	Merck Sharp and Dohme Research Labs.	1984-1987
Geraldine Schechter, M.D.	Veterans Administration Medical Center, Washington, D. C.	1985-1989

B. Biologic Response Modifiers Program (BRMP)

- Dr. Michael Chirigos retired from government service.
- Dr. Hsiang-Su Kung was recruited as Chief of the Laboratory of Biochemical Physiology.
- Dr. Cedric Long left as Section Head of the Procurement Formulation and Preclinical Trials Section to work in the Office of the Director, NCI, at the NCI-FCRF as General Manager of the Frederick Contract.
- Dr. Ronald Steis was recruited as Acting Chief, Clinical Research Branch. Drs. Jeffrey Clark, John McKnight, and John Smith were also recruited as physicians in the Clinical Research Branch.

- Dr. John R. Ortaldo was selected as the Chief of the Laboratory of Experimental Immunology.

C. Cancer Treatment Evaluation Program (CTEP)

- Dr. Dale Shoemaker was selected as the Chief of the Regulatory Affairs Branch.

- Dr. Dennis Cain joined the staff as Special Assistant to the Associate Director. Dr. Cain previously served in the Division of Extramural Affairs, NCI.

- Drs. Jean Grimm and Michaele Christian joined the staff of the Investigational Drug Branch as drug monitors, replacing Drs. Peter O'Dwyer and Brenda Foster. Dr. Grimm was recruited from the University of Wisconsin and Dr. Christian from Georgetown University.

- Two guest researchers joined the staff of the Investigational Drug Branch, each for one year. Dr. Alice Glover is here from the Department of Health in Australia, and Dr. Rita Liontto is visiting from the Instituto Nazionale in Italy.

- Dr. Roy Wu, formerly a Cancer Expert with the DTP, DCT, has been recruited into the Clinical Investigations Branch to serve as a Health Sciences Administrator.

- Ms. Kim Regan left the Office of the Associate Director to become the Executive Officer for the Division of Computer Research and Technology, NIH. Ms. Regan was the Administrative Officer for CTEP and then the Head, Program Analysis and Management Office.

D. Clinical Oncology Program (COP)

- Dr. Robert Makuch left the Biostatistics and Data Management Section to accept position as Associate Professor of Biostatistics at Yale University.

- Dr. Seth Steinberg from the EMMES Corporation joined the Biostatistics and Data Management Section as its Acting Chief.

- Dr. Tom Walsh joined the Pediatric Branch on IPA assignment from the Denver Children's Hospital.

- Drs. Frank Balis and Jim Hathorn joined the Pediatric Branch as attending physicians.

- Dr. Jack Roth, Head of the Thoracic Oncology Section of the Surgery Branch, left to head the Department of Thoracic Oncology at M.D. Anderson Hospital.

- Dr. Paul Sugarbaker, Head of the Colo-Rectal Section of the Surgery Branch, to head the Division of Surgical Oncology, University of Michigan.

- Dr. Harvey Pass has been appointed as Acting Head, Thoracic Surgery Section, Surgery Branch.

- Dr. Jim Yang joined the senior staff of the Surgery Branch.

- Drs. Peggy Findlay and Steven Hancock left the Radiation Oncology Branch in the fall of 1985.

- Dr. Robert Atcher left the Radiation Oncology Branch to head the Chemistry Section of Argonne National Laboratories.

- Dr. Tom Delaney joined the staff of the Radiation Oncology Branch.

- Drs. Antonio Fojo and Michael A. Bookman joined the Medicine Branch as attending physicians.

- Dr. Barnett Kramer from the University of Florida joined the senior staff at the NCI-Navy Medical Oncology Branch.

- Drs. Edward Sausville, Bruce Johnson, and James Battey joined the attending staff of the NCI-Navy Medical Oncology Branch.

E. Developmental Therapeutics Program (DTP)

- Dr. Arnold Welch left DTP to become Scientist Emeritus in the BRMP.

- Dr. Moreshwar Nadkarni retired from government service.

F. Radiation Research Program (RRP)

- Dr. John Antoine was recruited from the University of New Mexico to serve as Chief, Radiotherapy Development Program, RPP, and he is also serving as the Acting Associate Director of the Program.

International Treatment Research

International treatment research activities of the DCT are coordinated through the Office of the Director. Dr. Michael Friedman, Chief of the Clinical Investigations Branch, CTEP, has continued to coordinate the Japanese-American Bilateral Agreement. Three meetings were held during the past year. The first, in Tokyo, was entitled Progress of Combined Modality of Chemotherapy and Radiation Therapy; the second meeting, held in Hawaii, was on Clinical Trials Methodology: Chemotherapy and Biologic Response Modifiers; and the third, held in Bethesda, was on New Drug Development in Regional Chemotherapy.

The Italian and French bilateral agreements were administered by Dr. Gregory Curt. The French met with American colleagues in Paris this year to exchange information on AIDS. The Italian-American bilateral agreement met in Bethesda and three topics were discussed: recent developments in the molecular biology of cancer; tumor immunology applications to diagnosis and therapy; and new developments in cancer diagnosis and treatment of Hodgkin's disease.

The administration of the agreements with the People's Republic of China, Egypt and Poland was continued by Dr. Marcia Browne.

Most importantly, the agreements continued to sponsor individual exchanges of scientists on the laboratory-to-laboratory basis to enhance international exchange of techniques, expertise, and ideas.

Cooperative relationships with European cancer researchers and drug industries have been further strengthened by the Division's relationship with the EORTC (European Organization for Research on Treatment of Cancer). This interaction continues to have both preclinical and clinical components. In order to identify new agents useful in the treatment of cancer, drugs collected in Western Europe were screened by the Institut Jules Bordet in accordance with NCI protocols. In addition, important clinical programs are sponsored by this agreement. The EORTC has become a major testing ground for Phase I and Phase II agents. Continued exchange will allow the expedited access of the best new anticancer drugs on both sides of the Atlantic. The EORTC and NCI also sponsored a training program that began this past year and allowed postdoctoral fellows from Western Europe and Israel, as well as American colleagues, to spend significant research time in the laboratories of scientists in their area. Candidates will spend 3-5 years in sponsoring exchange laboratories before returning to their home institutions.

Scientific Accomplishments

1. Training

- In addition to the EORTC training program, the Office of the Director implemented a traineeship program in oncology nurses. Seven nurses successfully completed the first year of this program. This initiative is administered by Dr. Marcia Browne in close collaboration with the Nursing Department of the Clinical Center, NIH. An additional benefit of this nine-month training program was that six of the nurses were recruited by the Cancer Nursing Department after successful completion of this program and have become full-time staff nurses. It is expected that this program will expand and diversify to include a research fellowship for oncology nurses interested in the design and implementation of clinical trials. Training will include trial design, biostatistics, biochemistry, pharmacology, and immunology. It is hoped that this program will serve as a model for similar initiatives throughout NIH and the Nation. Because of the success of the first year of the program, this program will begin its second year in September with a class of 16 new graduate nurses.

- In addition, the Office of the Director coordinated a new fellowship program in biotechnology. This program, established under the training authority of the National Cancer Act, entitled Biotechnology Training Program, will allow American citizens to pursue new opportunities in molecular biology, genetic engineering, immunology, and molecular pharmacology.

2. Clinical Trials

- IL-2/LAK cell trials. The work of Dr. Steven Rosenberg and his colleagues in the Surgical Oncology Branch of DCT using adoptive immunotherapy for treatment of advanced cancers has continued this year. Dr. Rosenberg's treatment is based on the observation that exposing normal peripheral blood

lymphocytes to interleukin-2 (T-cell growth factor, IL-2) in vitro results in those cells developing the capacity to lyse a broad spectrum of tumors. Dr. Rosenberg has generated these lymphokine-activated killer cells (LAK cells) and reinfused them with IL-2 to promote their proliferation within the patient's bloodstream. Eighty-eight patients with advanced cancer had been treated through June 1, of whom 68 were evaluable; 31% of patients have responded to this therapy with either a complete response (CR) or a partial response (PR). The most responsive tumor appears to be renal cell carcinoma with 7 of 22 patients responding. Because of the promising nature of this therapy, six extramural centers were chosen to carry out a confirmatory trial using Dr. Rosenberg's technique to treat patients with renal cell cancer, melanoma, or colon cancer. At the present time, the patients needed for this trial are being accrued

- Hairy-cell leukemia trial. The use of alpha interferon in hairy-cell leukemia, a form of leukemia occurring in middle-aged males, has been studied by the Biologic Response Modifiers Program. Fifty-six patients have been treated, and over 90% of patients have either stabilized diseased, a PR, or a CR. A trial of deoxycoformycin, an inhibitor of adenosine deaminase, combined with alpha interferon has been initiated in an attempt to increase the CR rate in this disease.

- Advanced ovarian cancer. Forty-three patients with advanced ovarian cancer have been entered on a Medicine Branch trial using cytoxan, high-dose cisplatinum and radiation therapy; 67% of patients treated with 3-4 months of induction chemotherapy have achieved a clinical complete remission, and 65% of those have been pathologically free of disease at second-look surgery. The results thus far appear to achieve a higher frequency of pathological complete remissions than has been reported in the medical literature.

- Ewing's sarcoma. Thirty patients with Ewing's sarcoma, a common bone tumor of childhood, have been treated in the Pediatric Branch with intensive chemotherapy using vincristine, adriamycin, and cyclophosphamide and total body irradiation. The complete response (CR) rate is 100%. With a median follow-up of two years, 26 of the 30 patients are alive and 20 of 30 are free of disease. The initial CR rate is an improvement over our previous studies.

- Limited small-cell lung cancer. Ninety-seven patients with limited small-cell lung cancer have been randomized in a clinical trial in the NCI-Navy Medical Oncology Branch comparing the administration of intensive chemotherapy with or without the addition of aggressive radiation therapy to the primary tumor. The CR rate (81% versus 43%) and the local control of chest tumor strikingly favor the combined modality arm.

- Early Hodgkin's trial. The conventional approach to the treatment of patients with early-stage Hodgkin's disease involves the use of megavoltage radiation therapy. While the results of such an approach at several major treatment centers have been gratifying (78-88% long-term disease-free survival), the results from the patterns of care study reflecting a more conventional hospital experience are not so satisfactory (60-70% long-term disease-free survival). Furthermore, as many as 25-40% of patients relapse from radiation-induced complete remissions, and although many can be salvaged by chemotherapy, this is accomplished at a risk of induced second malignancies. Because of this, the Medicine Branch and the Radiation

ncology Branch of DCT are performing a randomized comparison between radiation therapy and MOPP chemotherapy in early-stage Hodgkin's disease. Thus far, 45 patients have been randomized to chemotherapy and 43 to radiation therapy arm with complete remission rates being 100% for combination chemotherapy and 95% for radiation therapy. With a median follow-up in excess of 32 months, 10% of the MOPP-treated patients have relapsed compared to 31% for those with radiation therapy. Disease-free survival and overall survival in randomized patients are significantly in favor of MOPP-treated patients.

3. AIDS

- A new anti-AIDS drug, 3'-azidothymidine, has produced improvement in T4 lymphocyte count in early Phase I-II trials conducted at the NCI.

- The NCI has established a formal collaborative effort with the National Institute of Allergy and Infectious Disease to develop effective therapies for AIDS. The NCI/DCT responsibility has been to coordinate and implement preclinical development of compounds to be tested clinically.

- The Developmental Therapeutics Program, in collaboration with the Food and Drug Administration, has developed an expedited preclinical toxicology process designed to bring promising anti-AIDS drugs to patients as rapidly as possible. This process focuses on generating animal data necessary for the determination of safe starting doses for human testing and minimizes redundancy in data collection.

- The discovery that compounds of the 2'3'-dideoxynucleoside class can effectively inhibit the growth of the AIDS virus in culture has led to the establishment of a major effort to synthesize effective analogs. Several compounds that have similar activity to the original compound, dideoxycytidine, have been synthesized by scientists at the NCI.

- In the Biologic Response Modifiers Program, scientists have discovered that brain cells have the same attachment proteins for the HTLV-III virus as found on the white blood cells infected by the AIDS virus (T4 lymphocytes). This finding may explain the propensity for HTLV-III infection of the brain.

- The development of a vaccine for the treatment of AIDS is being approached from several directions. In patients with early AIDS and AIDS-related complex, the objective will be to use passive immunization using synthetic peptides of HTLV-III antigens to arrest the progression of the disease by preventing spread of the virus to the non-infected T cells. Immunizations of patients with the virus vector for AIDS virus components is also being explored for high-risk individuals who do not have the disease.

4. Molecular Biology

- Among patients with small-cell lung cancer, those showing an amplified version of an oncogene called c-myc have significantly decreased survival as compared to patients whose oncogenes were not so amplified.

- During the past year, NCI investigators have made significant progress in understanding how estrogen promotes the growth of breast cancer. They have found that other hormones, known as polypeptide growth factors, help to carry out the cancer growth-stimulating functions of estradiol. This could be important in future detection and therapy of the disease because the growth factors (in particular, transforming growth factor alpha) must be secreted by the cancer cells in order to produce its effect. Future treatment could focus on blocking growth factor action. As a parallel line of investigation, it was found that antiestrogens induce the production of transforming growth factor beta (TGF-beta), itself a growth inhibitor of breast cancer. Even breast cancer cell lines that have lost the ability to respond to antiestrogen (which is a well-known clinical occurrence) still are growth inhibited by TGF-beta. Future treatment of breast cancer could take advantage of this and directly inhibit cancer cells with TGF-beta itself or with drugs that act in a similar fashion.

- It has been hypothesized that oncogenes may be important in pathologic differentiation of tumors and in defining treatment. An example of this has been found by Dr. Mark Israel in neuroblastoma, a tumor of neural crest origin occurring mainly in children as an adrenal mass. A variant of this tumor, peripheral neuroepithelioma, is histopathologically indistinguishable from childhood neuroblastoma. There are clinical differences, however. Peripheral neuroepithelioma occurs in adolescents, primarily as a mass in an extremity. Most importantly, this tumor is not responsive to the chemotherapeutic regimens used to successfully treat childhood neuroblastoma; thus the prognosis is poor. The chromosomal pattern of the peripheral neuroepithelioma differs from that of microscopically similar childhood neuroblastoma but is exactly comparable to another adolescent tumor, Ewing's sarcoma, a tumor of bone. In addition, the oncogene patterns of Ewing's sarcoma and peripheral neuroepithelioma, two microscopically distinct tumors, are very similar. When Pediatric Branch investigators apply treatments successful in Ewing's sarcoma to patients with peripheral neuroepithelioma, a high rate of response is obtained. These data suggest that the molecular footprints of a tumor may provide a better method to define effective therapies than does traditional microscopic examination.

5. Cell Line Screening Project

- A disease-oriented in vitro cell line screen for anticancer drugs is being established in the Developmental Therapeutics Program. Cell line panels of the following tumors will be included: melanoma, renal cell carcinoma, ovarian carcinoma, CNS tumors, prostate carcinoma, human leukemia, breast carcinoma, and multidrug-resistant lines of both the P388 and MCF-7 lines and non-small-cell and small-cell lung cancer lines. A preliminary trial of 100 randomly selected compounds in the new screen using human lung cancer cell lines showed that it is capable of identifying agents with selective effects against human cell lines; this finding gives considerable confidence that not only is the screen scientifically valid and workable but that the necessary selectivity will be achieved so that unmanageable numbers of compounds will not emerge as candidate drugs.

Special Initiatives

1. Cooperative Group Review (CTEP)

   Consultative groups of internists, radiation therapists, surgeons, and statisticians from the extramural/intramural community began reviewing the last seven years of cooperative group efforts. Testicular cancer and non-small-cell lung cancer were reviewed during FY 1986.

2. BRMP-CTEP Working Group Established

   A joint committee of four CTEP and two BRMP extramural scientists was established to encourage the submission of proposals and review the proposals submitted. The group works jointly with intramural scientists and with commercial firms to facilitate preclinical and clinical development of biologic response modifying programs.

3. Animal Review Committee Established

   The Animal Review Committee was established to monitor protocol compliance with the Guide to Care and Use of Laboratory Animals (PHS, revised 1985). All DCT protocols were reviewed to ensure compliance while meeting the investigators' appropriate needs for animal research.

4. Neutron Therapy Clinical Trials

   The neutron therapy contracts were initiated in 1979. Significant equipment design and development difficulties have been overcome with all four contractors now treating research patients. Patient treatments began at UCLA in mid-June 1986. The other contractors are M.D. Anderson Hospital, University of Washington, Seattle, and Cleveland Clinic. By the end of FY 1986, three Phase III neutron protocols will be accruing patients (prostate, head and neck, and lung). All contractors are increasing their research patient treatment accessions with Washington now experiencing a backlog of patients. In FY 1987 it is anticipated that the program will reach and maintain the projected capacity of 600 patients annually. Neutron therapy has been established as the treatment of choice in salivary gland tumors, and reported results appear favorable in local/regional prostate carcinoma.

Publications

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Definitions of Contract Groupings
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Drug Development

Compound Discovery - Preclinical contract program focusing on the discovery and screening of potential anti-cancer agents. Includes both natural products and synthetics, as well as testing (screening) *in vitro* and *in vivo*.

Compound Development - Includes data management, pharmacology and pharmacokinetics testing, formulation and analytical profiles, and toxicological protocols to meet FDA requirements.

Clinical Support - Preclinical contracts which provide direct support to the clinical trials program (excluding drug purchases).

Biologics Development

All non-clinical contracts administered by the Biological Response Modifiers Program, excluding contracts in support of the intramural program.

Diagnostic Imaging

Contracts administered by the Diagnostic Imaging Research Branch, RRP.

Radiation Development

Preclinical radiation contracts, including screening and synthesis of radiosensitizers and radioprotectors, dose calculations, and other preclinical radiotherapy contracts.

Clinical Trials

Drug Evaluation - Phase I, II, III drug development contracts administered by the Cancer Therapy Evaluation Program, including foreign clinical contracts in support of FDA requirements.

Biological Evaluation - Task order contracts for Phase I/II clinical trials of BRM's.

Radiotherapy - All clinical radiotherapy contracts administered by the Radiation Research Program.

Other - Contracts which support other research requirements throughout the Division of Cancer Treatment, including program support, data management for extramural contracts, and other technical support. Does not include intramural support contracts.

Support to Intramural

Contracts which support directly intramural research activities in the Developmental Therapeutics Program, the Clinical Oncology Program, and the Biological Response Modifiers Program.

Drug Purchases

The purchase of investigational agents used in DCT-sponsored Phase I/II/III clinical trials, where DCT provides the agents being used in the trial to both extramural and intramural investigators.

Program Management

Includes administration and dissemination of information to the medical and scientific community.

TABLE I

DCT Contract Program for FY1986
(Dollars in Thousands)

		FY 86 EST.	PERCENT
I.	Drug Development		
	A. Compound Discovery - Subtotal	$ 14,860	32%
	1. Acquisition	(3,285)	(7%)
	a. Natural Products	1,679	4%
	b. Synthesis	1,606	3%
	2. Screening	(11,575)	(25%)
	a. In Vivo (including animals)	8,921	19%
	b. In Vitro	2,659	6%
	B. Compound Development	7,385	16%
	C. Clinical Support	4,109	9%
II.	Biologics Development	2,540	6%
III.	Diagnostic Imaging	1,358	3%
IV.	Radiation Development	922	2%
V.	Clinical Trials - Subtotal	9,374	20%
	A. Drug Evaluation (Phase I/II/III)	5,700	12%
	B. Biological Evaluation (Phase I/II)	111	--
	C. Radiotherapy	2,218	5%
	D. Other	1,345	3%
VI.	Support to Intramural	3,329	7%
VII.	Drug Purchases	1,915	4%
VIII.	Program Management	362	1%
	TOTAL DCT CONTRACTS	$ 46,154	100%

TABLE II

DESCRIPTION OF CONTRACTS

IN THE

DIVISION OF CANCER TREATMENT

AEROJET STRATEGIC PROPULSION COMPANY (NO1-CM4-7584)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. The major effort (approximately 90%) of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities. The large-scale synthesis contracts are currently being recompeted under four separate RFP's.

ALABAMA, UNIVERSITY OF (NO1-CM2-7571)

This project is one of the three contracts whose objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improved bio-availability by increasing aqueous solubility; (b) increased compound stability; and (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles. This contract is being recompeted.

ALABAMA, UNIVERSITY OF (NO1-CM3-7631)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

ALBANY CARDIOTHORACIC SURGEONS (N43-CM5-7789) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This is an application for a Phase I contract in which we will evaluate the feasibility of using a new cancer treatment regimen in humans. The overall goal of this Phase I study will be to determine the toxicity and antitumor activity of this new regimen in rabbits.

The treatment regimen which we will evaluate is a two step process: 1) activation of systemic macrophages by intravenous inoculation of an effective macrophage activator such as BCG and 2) release of antitumor factors from the activated macrophages triggered by intravenous inoculation of detoxified endotoxin (monophosphoryl lipid A, henceforth abbreviated DE). Toxicity will be assessed by measuring physical, hemodynamic, hematological, chemical, and histological changes in rabbits after treatment. Antitumor activity will be assessed by monitoring post treatment serum for antitumor activity against murine and human cancer cell lines.

ALDRICH CHEMICAL COMPANY, INC. (NO1-CM4-7585)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities. The large-scale synthesis contracts are currently being recompeted under four separate RFP's.

ALLEGHENY-SINGER RESEARCH CORPORATION (NO1-CM3-7512)

This contract is expected to develop the criteria, guidelines and procedures for the proper use of the equipment representing the major heat generating modalities (radiofrequency, microwave, and ultrasound) and the ancillary equipment necessary for the treatment of cancer with heat. The criteria and guidelines developed will be utilized to initiate a quality assurance and assessment program in hyperthermia. This Contractor is expected to implement and conduct such a program in the latter years of the contract.

AMERICAN BIOTECHNOLOGY CO. (N43-CM5-7790) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Although investigation into biological response modifiers has increased recently, the majority of effort has been towards potentiating the hosts cell mediated immunity. Data accumulated by our laboratories indicates that the methylfurylbutyrolactone (MFBL) class of compounds represent new synthetic immune response modifiers capable of activating several components of immune response with negligible toxicity to the host. This proposal describes research designed to analyze one aspect of the immunostimulatory activity of MFBLs, the ability to stimulate antibody production. Phase I research will concentrate on the optimization of treatment regimens and the evaluation of the range of activity of the drugs toward the primary and secondary antibody response to T-dependent and T-independent antigens. We further intend to study the effect of MFBL in vivo on the improvement of the quality and quantity of antibody production in mice as it relates to monoclonal antibody technology. In addition, we will assess murine in vitro antigen sensitization and its potential application to human hybridoma technology.

AMERICAN BIOTECHNOLOGY CO. (N43-CM5-7791) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This project involves determining the ability of a new class of synthetic biological response modifiers (BRM) known as methylfurylbutyrolactones (MFBL) to augment the production of lymphokines (IL-2, B-Cell Growth Factor, and immune

interferon) and monokines (IL-1, prostaglandins, and cytolytic factor). Our laboratory has determined that the MFBL's are potent immunostimulatory compounds. MFBL's are capable of increasing the following immune functions: (1) lymphocyte blastogenesis in response to polyclonal mitogens; (2) primary and secondary antibody production; (3) phagocytosis by both macrophages and PMN's. It is known that the above mentioned immune functions are regulated in some way by lymphokines and/or monokines. Therefore, we plan to investigate the possibility that MFBL's enhance these immune functions by stimulating an increased production of lymphokines and/or monokines. This hypothesis will be tested both *in vivo* and *in vitro* using established murine lymphokine and monokine assay systems.

AMERICAN BIOTECHNOLOGY CO. (N43-CM5-7793) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The goal of this project will be to screen new derivatives of L-ascorbic acid adducts classified as methylfurylbutyrolactones, also referred to as Nafocare B. Working in collaboration with a synthetic organic chemistry group at West Virginia University, we have determined that the parent compound, MFBL, has extensive immune potentiating activity. Our project will analyze five new derivatives of L-ascorbic acid in an attempt to verify which prosthetic groups other than the butyrolactone structure are critical to biological activity. In addition, we hope to discover new synthetic immune biological response modifiers. All test compounds will be screened presumptively using the mouse lymphocyte stimulation assay. If the compound is positive for activity further confirmatory immunoassays will be performed using T-cell, B-cell, and macrophage effector cells.

APPLIED GENETICS, INC. (N43-CM5-7847) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A group of human tumor cell strains has been identified which is more sensitive to growth inhibition produced by human interferon than are other tumor cell strains or normal human fibroblasts. This group of tumor strains, designated the Mer$^-$ phenotype, shares a biochemical defect in DNA repair. These strains may prove useful as the basis for a rapid, sensitive and reliable bioassay for antitumor activities of manufactured interferons. Results from Phase I research are expected to establish measures for interferon inhibition of cell growth, colony forming ability, DNA synthesis, and ornithine decarboxylase activity in several Mer$^-$ strains.

ARBUS (N43-CM5-7815) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this SBIR contract was to establish feasibility for a computerized pharmaceutical tracking system for monitoring the manufacture and quality of investigational products used in the Division of Cancer Treatment. This contract expired March, 1986.

ARIZONA, UNIVERSITY OF (NO1-CM1-7522)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Center for Devices and Radiological Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

ARIZONA, UNIVERSITY OF (NO1-CM3-7621)

Difficult dosage form development projects are assigned to this Contractor for evaluation. This Contractor has particular expertise with cosolvent approaches to improve drug solubility. Several novel solvents are being evaluated in an attempt to improve the options available to formulate poorly water soluble and unstable compounds. This Contractor is also responsible for the preparation of pilot batches and chemical analysis of these novel formulations. This contract expired June, 1986.

ARIZONA, UNIVERSITY OF (NO1-CM5-7662)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as anti-tumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

ARTHUR D. LITTLE, INC. (NO1-CM3-7596)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor studied Deoxyspergualin (NSC 356894). The contract was conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. The information was used for IND filing and by Phase I investigators in determining appropriate schedules for administration. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

ASH STEVENS, INC. (NO1-CM4-7586)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts and/or quality required. About 60% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities. The large-scale synthesis contracts are currently being recompeted under four separate RFPs.

BANNER GELATIN PRODUCTS CORPORATION (NO1-CM1-7402)

The objectives of this contract are to provide facilities and capabilities for the development and production of soft gelatin capsules containing investigational anticancer agents. This Contractor is responsible for conformity to U.S. FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. All products are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to

clinical investigators. This contract expired June, 1986, and will not be recompeted.

BATTELLE MEMORIAL INSTITUTE (NO1-CM1-7365)

This service type Prime Contract with Battelle Memorial Institute is for management of subcontractors performing the toxicological evaluation of potential oncolytic agents, biologic response modifiers, and other modalities. Through the Prime Contract mechanism, preclinical toxicology studies of agents under consideration for clinical use are handled under a single management-type contract. The work scope under this contract is comprised of four tasks as follows: Task I - protocol studies; Task II - high priority toxicity studies (i.e., any portion of the Protocol of the Toxicology Branch); Task III - organ specific toxicity testing; and Task IV - automation of toxicity data, anomaly detection, scheduling of studies, and financial management. This is the fourth of a five year contract.

BATTELLE MEMORIAL INSTITUTE (NO1-CM4-7648)

This contract is for the in vivo screening of new materials in the P388 leukemia pre-screen and for the secondary testing of materials of interest to the Developmental Therapeutics Program. The secondary testing may consist of testing in both a panel of murine tumor models and a human xenograft tumor model. Testing is conducted at a level of approximately 18,000 L1210 equivalents per year. Special studies for the detailed evaluation of compounds of Program interest, as well as studies with new tumor panel systems, are conducted upon request of the Project Officer.

BEN VENUE LABORATORIES, INC. (NO1-CM2-7508)

This resource contract provides for the development and production of parenteral clinical dosage forms of anticancer agents. This Contractor has the capacity for preparing production batches of liquid-filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of parenteral products; (2) production of sterile products; and (3) quality assurance testing of finished products. All products are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BEN VENUE LABORATORIES, INC. (NO1-CM5-7595)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the Division of Cancer Treatment. This Contractor is responsible for conformity to U.S. FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. All products are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

BIOLOGICAL THERAPEUTICS (N43-CM5-7761) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The long-term objective of the proposal is to create and evaluate the effectiveness of monoclonal antibody directed against the receptor on malignant cells recognized by activated macrophages. Macrophages, when activated, recognize and

destroy malignant cells. Activated macrophages do not recognize non-malignant cells. The nature of this receptor site in unknown. In the current Phase I proposal, using novel immunization protocols, monoclonal antibody will be produced to that recognition site. The antibody will be screened in appropriate assays for reactivity with malignant and normal cells, inhibition of binding, and immunomodulation.

BIOLOGICAL THERAPEUTICS (N43-CM5-7796) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The long-term objective is to prepare an effective liposome encapsulated reagent which will be preferentially targeted to liver macrophages resulting in macrophage activation for the therapy of metastatic hepatic disease. The specific aims are: (1) to produce a lipophilic derivative of the mycobacterial cell wall and incorporate that in liposomes designed to be preferentially taken up by the hepatic macrophage; (2) to determine the toxicity of the reagent; (3) to determine the effects on human monocytes in vitro; (4) to determine the effects on hepatic metastasis in several animal models; and (5) to determine the toxicity and immunological effects in Phase I-II clinical trials.

BIOMEDICAL RESEARCH LABS. (N43-CM5-7765) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Anti-idiotypic monoclonal antibody (AIMA) therapy has curative potential for low grade B cell lymphomas through immunologic down regulation of the malignant idiotypic clone as well as antibody dependent cell mediated cytotoxicity. We propose to test the feasibility of (1) preparing anti-idiotype in a commercial laboratory and (2) improving the idiotype harvest as well as shorten the production interval by transforming malignant B cells with Epstein Barr Virus prior to hybridization.

BIOTECH RESEARCH LABORATORIES (NO1-CM3-7558)

This SBA contract was initiated in 1983 to provide assistance to the Developmental Therapeutics Program (DTP), Drug Evaluation Branch (DEB) in maintaining an orderly flow of materials to screening laboratories, evaluating the test results, scheduling additional testing as appropriate and coordinating the data entry to files. The existing contract expired May, 1986 and is being recompeted as a broad services effort with a modified workscope designed to support the entire DTP. In view of this anticipated broadening of the requirements, during the 1985-86 period the contract was transferred from the DEB to the newly-formed Office of Extramural Resource Analysis and Development which has responsibility for the overall coordination of the DTP extramural resources in contracts, grants and cooperative drug discovery groups.

BIOTECH RESEARCH LABORATORIES (NO1-CM3-7590)

This contract supplies the Government with well-characterized normal and neoplastic mammalian tissue culture cells and receives, processes, and distributes fresh human leukemic cells and tissues. Complete records are maintained on all biological materials handled under the contract.

BIOX, INC. (N43-CM5-7783) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The discovery of oncogenes, genes which cause cancer, has had a major impact on research and potential diagnosis and treatment of cancer. Some of the products of oncogenes have been found to be similar to growth factors and growth factor receptors with regard to biological activities and amino acid sequences. In Phase I of this proposal, monoclonal antibodies will be generated to prototype oncogene products (epidermal growth factor, EGF, platelet derived growth factor, PDGF) as well as other available proteins. These monoclonal antibodies will be tested against a panel of antigens to characterize their specificities and cross reactivities.

BIOX, INC. (N43-CM5-7788) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Infection by herpes simplex virus (HSV) has been associated with cervical cancers. In addition, neonatal infection by HSV is oftentimes fatal. The currently recommended procedures for screening pregnant females in the high risk category (i.e., history of active or recurrent HSV infection) include weekly virus cultures. These procedures are costly, time-consuming and relatively insensitive. In contrast, a reliable and rapid immunocytological test employing monoclonal antibodies for detecting HSV-infected cells in cervical smears could be carried out at a considerable cost-saving. The Phase I effort will emphasize the development and selection of suitable monoclonal antibodies for detecting expression of HSV gene products and for differentiating between infection by HSV-1 and HSV-2. In vitro virus-infected cells will be employed for selecting type-specific and cross-reactive monoclonal antibodies and for establishing optimal conditions for cell fixation and staining (i.e., immunofluorescence or immunoperoxidase).

BOWMAN GRAY SCHOOL OF MEDICINE (NO1-CM4-7564)

This is one of five contracts awarded in May, 1984 (1) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (2) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, lung cancer, liver metastases, and congenital heart disease. They have also provided coordination for the CWG of the development of the lung cancer protocol. Patient imaging has begun in each of these studies.

BRISTOL LABORATORIES (NO1-CM3-7556)

The objective of this fermentation contract is to discover and isolate novel antitumor agents from microbial sources. This contract involves: (1) isolation of unique and unusual microorganisms; (2) pre-screening the cultures for biological activities using microbial assays and tumor cell cultures; (3) secondary screening of selected cultures using P388 in vivo assay; (4) dereplication of the active components to determine their novelty; (5) chemical isolation, characterization and evaluation in vivo of the novel agents; and (6) optimization and scale-up production of large quantities of the novel agents for thorough evaluation in DCT screens. The culture isolation, in vitro screening and initial

in vivo screening are carried out at the Bristol Myers Research Institute in Tokyo while the dereplication, further in vivo evaluation, chemical isolation and characterization are carried out in the Syracuse laboratories. The dereplication technique has been fully automated to effectively select only novel chemotypes. Bristol is currently concentrating on three of their four top leads: Elsamicin has passed DN2A. Preclinical toxicology is currently being done at Bristol. A highly potent compound which is active against P388 in vivo down to 25 nanogram levels has been successfully produced in gram quantities under a separate contract between Bristol and FCRF. Formulation studies and structure elucidation of this compound are on-going. Another compound has been formulated and pilot toxicological and pharmacokinetic studies are underway. This contract terminated March, 1986.

BSD MEDICAL CORPORATION (N43-CM5-7840) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor has begun development of a regional hyperthermia heating applicator which is referred to as the "thin-shell annular phased array." This Contractor would like to use this device in association with a CT scanner to measure the temperature distributions (do non-invasive thermal mapping) during hyperthermia treatment. The purpose of the Phase I contract will be to test the feasibility of measuring temperature distributions with the CT scanner using test phantoms and determining the extent and significance of artifacts due to the "thin-shell annular phased array."

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7672)

On this contract, several immunoconjugates were evaluated in vitro. These included cytotoxic drug conjugates to T101 monoclonal antibody. A clinical Phase I trial will be initiated using a T101-ricin-A-chain immunoconjugate. This contract expired June, 1986.

CALIFORNIA, UNIVERSITY OF (NO1-CM4-7684)

This is one of five contracts awarded in May, 1984 (1) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (2) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, brain neoplasms, and congenital heart disease. They have also provided coordination for the CWG of the development of the uterine neoplasms and congenital heart disease protocols. Patient imaging has begun in each of these studies as well as those in lung cancer.

CALIFORNIA, UNIVERSITY OF (NO1-CM5-7708)

The objective of this collaborative effort is to develop recommendations and guidelines for a program in Interstitial Radiotherapy. The recommendations shall include calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of

interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and after-loading procedures where appropriate.

CALIFORNIA, UNIVERSITY OF (NO1-CM5-7710)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as antitumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

CALIFORNIA, UNIVERSITY OF (NO1-CM9-7315)

This contract provides for a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support clinical neutron therapy research at UCLA. The facility has been constructed on the grounds of the Wadsworth Veterans Administration Medical Center near the UCLA campus in Los Angeles. Construction of the facility began in November, 1982 and was completed in March, 1985. Phase II construction was completed in March, 1986. The cyclotron and associated components of the neutron therapy system were to be fabricated and assembled by The Cylotron Corporation in Berkeley, CA. However, the company filed for bankruptcy under Chapter 11 of the Bankruptcy Code while the cyclotron system for UCLA was being built. The various components of the cyclotron were then moved to the VA hospital facilities for final assembly, fabrication and testing. This work is nearly complete and patient treatments are expected to begin in early summer 1986.

CALIFORNIA HEALTH CONNECTION (N43-CM5-7781) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Tumor necrosis factor (TNF) is a 17,000 dalton polypeptide secreted by macrophages which has anti-tumor cytolytic activity. TNF does not appear to affect normal cells and has been shown repeatedly to either kill or prevent the growth of tumor cells in both in vitro and in vivo model systems. TNF is thought to exert its effect through a process initiated by the binding of TNF to specific cell surface receptors. The long-term objective of this proposal is to produce monoclonal antibodies specific for the TNF receptor. Such antibodies will then be used to (1) purify large quantities of receptor for use in in vivo anti-tumor immunity studies, (2) produce highly tumor-specific antibody-toxin/radiolabel conjugates for cancer diagnosis and therapy, and (3) study the structure and function of the receptor-TNF complex. Phase I studies will attempt to identify a specific TNF receptor by (1) cross-linking labelled TNF to cell surface receptors with subsequent solubilization and visualization of the TNF receptor complex, and (2) TNF-agarose affinity chromatography of solubilized labelled cell membrane proteins.

CALIFORNIA HEALTH CONNECTION (N43-CM5-7826) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this contract was to evaluate the response of human breast cancer cells and normal breast epithelial cells to methotrexate using the following two in vitro approaches: a cloning assay which does not use serum or a fibroblast feeder layer and an assay using a FACS (fluoroescence activated cell sorter) analysis. Existing assays for breast cancer cells cannot determine methotrexate sensitivity because medium components rescue sensitive cells. This contract expired March, 1986.

CARCINEX, INC. (N43-CM5-7762) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Carcinex, Inc. is a small business corporation funded to develop and market new methods to diagnose, stage and treat squamous cell carcinomas. Carcinex has already developed 20 murine monoclonal antibodies which react with antigens present on squamous cell carcinomas which are not found on normal squamous epithelium. The goals of this proposal is the isolation of transforming genes present in squamous cell carcinoma of the head and neck in order to develop new molecular reagents for the diagnosis and treatment of these disorders. In Phase I of this proposal, we will transfect the DNA of 20 human tumor cell lines established from squamous cell carcinomas of the head and neck into the NIH 3T3 cell and into explants of normal squamous cells of head and neck epithelium to isolate cellular transforming genes which play a role in the evolution of these malignancies.

CARCINEX, INC. (N43-CM5-7764) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of the proposed research is to conjugate the anti-cancer agents methotrexate (MTX) and cisplatin (CP) onto anti-squamous cell carcinoma (SCC) antibodies and to compare the therapeutic efficacy of the drug-antibody conjugates to unconjugated drugs and antibodies. Carcinex has developed over 40 highly tumor associated murine monoclonal antibodies (MoAb) against human SCC tumors. Phase I goals are to: (1) conjugate several anti-human SCC MoAbs with MTX and CP; (2) test in vitro cytotoxicity of MoAb-conjugated vs. unconjugated drugs using human SCC cell lines; (3) test in vivo toxicity of MoAb-conjugated vs. unconjugated drugs using nude and conventional mice; and (4) test in vivo efficacy of MoAb conjugated vs. unconjugated drugs in preventing growth of human SCC tumors in nude mice.

CENTER FOR DEVICES AND RADIOLOGICAL HEALTH, FDA (Y01-CM2-0107)

This Interagency Agreement provides technical support to NCI and to the five contractors participating in the collaborative Phase I evaluation of equipment for the hyperthermic treatment of cancer. The Division of Physical Sciences, Center for Devices and Radiological Health, Food and Drug Administration, has a number of highly recognized experts in electromagnetic radiation and in ultrasound who are available on a consultative basis to assist in the hyperthermia research program.

CHARLES RIVER LABORATORIES (NO1-CM3-7526)

This contract provides for the rederivation of approximately 16 mouse and rat strains and two guinea pig strains on an annual basis. Rederived strains will be distributed to genetic centers for expansion and replacement of producing strains.

CHARLES RIVER LABORATORIES (NO1-CM3-7626)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities. This contract effort is currently being competed.

CHARLES RIVER LABORATORIES (NO1-CM5-7653)

This Rodent Production Center contract, located in North Carolina, produces hybrid mice and nude mice in a maximum barrier environment.

CHARLES RIVER LABORATORIES (NO1-CM8-7212)

This contract provides for the continual monitoring of the associated isolators within the DCT animal program to determine the flora status of these foundation isolators and to check for specified isolator contaminants. In addition, this contract will provide the correct organisms for establishing flora in new isolators. This contract terminated August, 1986. This effort will be performed on a smaller scale through the AHDL at FCRF.

CHEUNG LABORATORIES, INC. (N43-CM5-7838) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this Phase I contract is to determine the feasibility of using phase and amplitude controlled multiple microwave applicators for improving the power disposition patterns, i.e., the specific absorption rate (SAR), when inducing hyperthermia in deep seated tumors by electromagnetic means. The problems associated with exercising control (beam steering) over the amplitude and relative phases of multiple electromagnetic sources will be determined and clarified in greater detail to determine the research and development needed to make such a system functional.

CHROMATOCHEM, INC. (N43-CM5-7837) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The overall objective of the Phase I research is to develop an automatable molecular radiation dosimeter which would measure radiation induced damage to cellular DNA. The assay techniques to be used will involve extraction of the DNA from irradiated cells and high performance liquid chromatographic measurement of thymidine diol content. The sensitivity goal for the HPLC assay is detectability of 0.1 picomole of thymidine diol in DNA from samples of 10^7 cells.

CIVILIZED SOFTWARE (N43-CM5-7811) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A total of five SBIR contracts covering two topics in Phase I have been managed by the Information Technology Branch, DTP, during this reporting period. This Contractor is to design a microcomputer-resident version of MLAB, an interactive mathematical modelling package.

CLEVELAND CLINIC (NO1-CM4-7673)

This Contractor will study the effects of recombinant alpha interferon and anti-melanoma antibody, R24 in patients with malignant melanoma. This monoclonal antibody is directed against the sialoganglioside, GD_3, which is preferentially displayed on the surface of most malignant melanoma cells. Clinical toxicity and biologic response from this combination will be monitored closely. This trial began in the Spring. This contract will expire December, 1986.

CLEVELAND CLINIC (NO1-CM4-7685)

This is one of five contracts awarded in May, 1984 (1) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (2) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, lung cancer, brain neoplasms, and congenital heart disease. They have also provided coordination for the CWG of the development of the cervical myelopathies protocol and for the continuing coordination of the brain neoplasms protocol. Patient imaging has begun in each of these studies.

CLEVELAND CLINIC (NO1-CM5-7714)

This Contractor is part of a collaborative working group to conduct Phase III randomized clinical trials and Phase II studies of fast neutrons versus photons for tumors which are not presently controlled by conventional radiotherapy in the major anatomical sites. The working group meets twice each year to design protocols, review the quality control of each contractor through subcommittees, and collaborate with other neutron contractors on state-of-the-art neutron treatment techniques. Phase III protocols are being opened for randomized studies to compare neutrons with photons for 1) patients with squamous cell or lympho-epithelioma carcinoma of the upper aero-digestive tract, 2) adenocarcinoma of the prostate gland, and 3) cancer of the uterine cervix.

COLLABORATIVE RESEARCH, INC. (N43-CM5-7767) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Lymphotoxin is a lymphokine which has potent anti-tumor activity. It is a glycoprotein with a molecular weight of 25,000 daltons. Due to the minute amount produced by lymphocytes, evaluation of its pharmaceutical potential requires that it be cloned and expressed in a suitable host. In order to obtain the correct glycosylation pattern, we propose to express it in mammalian cells. In Phase I, we plan to clone the full length lymphotoxin cDNA. Peripheral blood lymphocytes

will be used for poly(A) RNA isolation from which cDNA will be synthesized. The vector we chose to use for establishing a cDNA library is the single stranded phage f1. The cDNA library will be screened with chemically synthesized oligonucleotides. The full length cDNA will be assembled with overlapping recombinant clones if necessary. It will also be sequenced. In addition, the activity assay for lymphotoxin will be set up in Phase I.

COLLABORATIVE RESEARCH, INC. (N43-CM5-7768) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this research plan is to isolate a cDNA sequence encoding tumor necrosis factor (TNF) and, by manipulation of that sequence, to produce and secrete the mature TNF protein from the yeast Saccharomyces cerevisiae. Previous work on TNF has shown it to have cytostatic or cytolytic activity in vitro against a number of transformed cell lines and to have tumor necrotic activity against various tumors in animal models. Most importantly, it has no adverse effect on normal cells either in culture or in animal models. This specificity for tumor cells suggests a potential valuable therapeutic role for TNF in treating human tumors. In order to establish its value, however, sufficient quantities of this rather rare protein must be isolated for testing. The overall aim of this research proposal is to produce TNF economically in large quantities from yeast cells.

COLLABORATIVE RESEARCH, INC. (N43-CM5-7792) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

We propose to develop methods for the large scale production of human colony stimulating factors (CSF) from normal human peripheral blood lymphocytes. The fermentation methods employed will be similar to those used for the production of natural Interleukin-2. We are producing human IL-2 for clinical evaluation by the Biological Response Modifiers Program (BRMP). Purified CSFs will be tested in preclinical screen and eventually used for efficacy studies for accelerated restoration of suppressed bone marrow function in chemotherapy. The Phase I suitable assay methods utilizing normal human bone marrow and soft agar culture techniques will be developed. Fermentation conditions will be optimized to promote production of CSF. Attempts will be made to maximize production of G-M or Multi-CSF since these factors are of highest clinical interest.

COLLABORATIVE RESEARCH, INC. (N43-CM5-7848) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The long-term objective of this project is to develop processes for the production of B-cell growth factor (BCGF) and to test the potential applications of BCGF for the treatment of human immunopathological conditions. BCGF may be useful as an immunotherapeutic agent in the treatment of primary immunodeficiency diseases and possibly in certain B-cell leukemias. The principal goal of the first phase of this project is to develop a continuous B-cell line which can be used to facilitate the large scale purification of BCGF. Freshly isolated B-cells from normal human donors will be placed into an initial suspension culture. Cultured cells will then be cloned in soft agar and recloned by limiting dilution. B-cell lines will be maintained with partially purified BCGF already developed at Collaborative Research. It is anticipated that a BCGF-dependent cell line will be developed during Phase I. This line will be used to develop a simple, rapid and reproducible assay for the detection of BCGF.

COLUMBUS BIOMEDICAL RESEARCH INC. (N43-CM5-7824) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Phase I SBIR contract involved preliminary studies directed toward development of an artificial, drug containing vitreous for treatment of ocular melanoma. Studies were directed toward confirming the ocular compatability and vitreous replacement feasibility of the gels, optimization of drug release from these gels in vitro, and development of prototype formulations. This contract expired March, 1986.

COMPUTER TECHNOLOGY AND IMAGING, INC. (N43-CM5-7806) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this contract is to develop a new two-dimensional position encoder for Positron Emission Tomography (PET) with position resolution better than 3 millimeters. The improved position resolution (< 3 mm) will allow PET tomographs to obtain the theoretical limit imposed by the momentum of the positron. It also offers a reduction in front end cost.

COMPUTER TECHNOLOGY AND IMAGING, INC. (N43-CM5-7835) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objectives of this contract are: (1) to refine the synthesis of ^{18}F-2-FDG from an ^{18}O target for use in positron tomography with the company's 11 MeV proton cyclotron currently under construction; (2) to utilize the Tewson synthesis of ^{18}F, namely nucleophilic attack on the mannose cyclic sulfate ester; (3) to obtain higher yield with a lower starting activity as well as freedom from the labeled fluoromannose isomer; and (4) to make the synthesis process semi-authomatic to automatic with micro-processor hardware modifications and new software.

COMPUTER TECHNOLOGY AND IMAGING, INC. (N43-CM5-7836) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The major aims of this contract are to design, fabricate and test an automated chemistry unit that: (1) synthesizes ^{15}O - labeled butanol used in cerebral blood flow measurements rapidly (less than 5 mins.) and efficiently, and (2) mounts on the company's small medical accelerator.

COMPUTER TECHNOLOGY AND IMAGING, INC. (N43-CM5-7844) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This contract will test, under actual operating conditions, the proposed basic modeling and calculations underlying the design of a self-contained shield for a clinic-based accelerator for the production of positron emission radioisotopes. A final specification for a neutron shield will be developed in light of experimental results so as to attenuate the exposure to a level consistent with the goal.

CONCEPT & DESIGN, INC. (N43-CM5-7849) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A total of five SBIR contracts covering two topics in Phase I have been managed by the Information Technology Branch, DTP, during the reporting period. This Contractor is to design a microcomputer-resident version of MLAB, an interactive mathematical modelling package.

CONNECTICUT, UNIVERSITY OF (NO1-CM5-7692)

This contract is directed toward the fermentation of fungal isolates to provide NCI with lyophilized broth and cell extracts to be evaluated for antitumor activity. The contract involves: (1) isolation of various genera of fungi; (2) fermentation in three media and harvesting at two or more stages of the growth cycle; (3) lyophilization of filter-sterilized broths; (4) extraction of the cell mass; and (5) optimization and scale-up of fermentation of cultures selected by NCI. There are 500-600 cultures currently in their collection which is represented by various fungal types including basidiomycetes, ascomycetes, zycomycetes and deuteromycetes.

DELTA RESEARCH GROUP, INC. (N43-CM5-7800) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor is developing a digital recording capability for medical imaging not commercially available in any product today. Specifically, they plan: (1) to design the source for short wave length response media from currently available sources or from new experimental sources; (2) to specify the source for narrow gap high frequency magnetic recording heads; (3) to select appropriate tape transport for modification from a variety of existing products; (4) to measure short wave length responses of various media; and (5) to measure drop out response of the media.

DIGIRAD CORPORATION (N43-CM5-7802) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this proposal is to develop an energy selective x-ray detector that can be utilized with standard radiographic equipment. The resulting image could be improved through selective tissue subtraction and could improve diagnosis of these lesions through tissue characterization. Specific aims would be to use computer similations to design an optimal energy selective detector based on photostimulable phosphor technology, construct a prototype, and verify performance by use of phantoms.

DOTY SCIENTIFIC, INC. (N43-CM5-7804) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Highly specialized electronic improvements have been demonstrated in the development of high power Class AB broadband radiofrequency (rf) pulse amplifiers such as are used in the circuits of magnetic resonance imaging systems for medical diagnosis. Examples include high performance rf amplifiers for very low impedance, which provide minimum distortion at 200 watts over a frequency bandwidth of 8-700 MHz and 5 kilowatts over 5-300 MHz.

DUKE UNIVERSITY (NO1-CM4-7686)

This is one of five contracts awarded in May, 1984 (1) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the noninvasive characterization of tissues, and (2) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases,

brain neoplasms, lung cancer, and congenital heart disease. They have also provided coordination for the CWG of the initial development of the brain neoplasms protocol. Patient imaging has begun in each of these studies.

DYNAMAC CORPORATION (NO1-CM3-7563)

The objective of this project is to develop and maintain a systematic literature surveillance effort to identify published compounds which warrent acquisition based on their structural characteristics and biological properties. This contract is monitoring a broad base of chemical biochemical, biological and patent literature to identify compounds for potential acquisition or task order synthesis. This contract is being recompeted.

EAGLE-PICHER INDUSTRIES (NO1-CM3-7634)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

EG&G MASON RESEARCH INSTITUTE (NO1-CM4-7647)

As the result of budgetary restraints, this contract was reduced during the current contract period and terminated June, 1986. This contract has provided for in vivo testing in the P388 leukemia pre-screen, for the evaluation of materials in specified tumor panel models, for detailed evaluations as requested by members of the NCI staff, and for evaluations of tumor models as directed by the Project Officer. In addition to the P388, other tumor systems currently being utilized include the murine L1210, M5076 sarcoma, and B16 (being phased out). Xenograft models include the colon, breast and lung sub-renal capsule models, the LOX amelanotic melanoma and the HL-60 which is used for special testing of differentiating agents.

EMMES CORPORATION (NO1-CM1-7371)

This contract provides operations office support for the Gastrointestinal Tumor Study Group and the Intergroup Testicular Cancer Studies. Functions include coordination of protocol development, editing and preparing final form of protocols, forms design, randomization, quality control of data, editing and preparing meeting agenda which include clinical trials reports, writing and preparing minutes of meetings, preparing correspondence, recordkeeping, and files maintenance. This contract also provides research and information management assistance to the Cancer Therapy Evaluation Branch in support of its mission of coordination of ongoing and planned clinical trials. This Contractor will be offered a new contract as a result of the recompetition of this project.

EMMES CORPORATION (NO1-CM8-7193)

The EMMES Corporation provides the statistical support for the Gastrointestinal Tumor Study Group. They assist in design of protocols, perform statistical analyses of studies, and assist investigators in preparing manuscripts presenting the data. This contract expired August, 1986.

ENERGY, DEPARTMENT OF (LAWRENCE BERKELEY LABORATORY) (YO1-CM2-0110)

This contract contributes heavy ion data to a collaborative effort to improve treatment planning with heavy particles, to include protons, helium and heavy ions, and neutrons. In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, each contractor contributes patient data to other contractors that are members of the collaborative working group. Treatment plans developed by each contractor using the same patient data will be compared using evaluation methods developed by the group. A table of normal tissue tolerances for various organs has been developed by the working group.

FEIN-MARQUART ASSOCIATES, INC. (NO1-CM5-7689)

This contract provides for the operational support of the Drug Information System (DIS), a large computerized system that supports the day-to-day activities of the Developmental Therapeutics Program. This system maintains databases containing chemical information, material inventory information, shipping histories, and the results of activity testing. The system further allows the interactive interrogation of these databases, provides information on the progress of chemicals through the testing procedures, prints letters requesting chemicals and acknowledging their receipt, etc. Under this contract, remedial fixes are performed where necessary, and assistance is provided to NCI personnel for the resolution of problems experienced in the operation of the system. Under this contract, also, the biology module has recently been completed, and is being tested prior to incorporation into the DIS.

FEIN-MARQUART ASSOCIATES, INC. (N43-CM5-7812) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A total of five SBIR contracts covering two topics in Phase I have been managed by the Information Technology Branch, DTP, during the reporting period. This contract calls for the design of a new version of the Drug Information System that would run on a 32-bit computer.

FEIN-MARQUART ASSOCIATES, INC. (N43-CM5-7813) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this Phase I SBIR contract was to establish feasibility for a computerized pharmaceutical tracking system for monitoring the manufacture and quality of investigational products used in the Division of Cancer Treatment. This contract expired March, 1986.

FLOW LABORATORIES, INC. (NO1-CM1-7398)

This resource contract provides the Division of Cancer Treatment with storage and distribution capabilities for the large volume of investigational and commercial drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. This contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs, and the prompt removal of expired materials. This contract is currently being recompeted.

FLOW LABORATORIES, INC. (NO1-CM2-7505)

This Contractor furnishes the National Cancer Institute with facilities and services for the storage and distribution of synthetic chemicals, bulk chemical drugs and crystalline natural products. Samples are weighed, packaged and shipped to contract screening laboratories and also to various domestic and foreign research institutions. This contract also provides for the maintenance of accurate inventory records. This is an on-going operation and supports all of the DTP programs.

FOX CHASE CANCER CENTER (JEFFERSON MEDICAL CENTER) (NO1-CMO-7432)

This contract is being conducted at Jefferson Medical Center, under a subcontract from the Fox Chase Cancer Center. It was designed to study the Phase I effects of decreasing the number of circulating suppressor T cells in patients with disseminated malignancy by the infusion of anti-T suppressor cell antibody (anti-Leu 2a, Becton-Dickinson). Doses of 1, 5, 25, 100, and 500 mg have been administered by infusions lasting from 2 hours to 24 hours. Twenty patients have been accrued to this protocol. Pharmacokinetic data and immunologic data are being evaluated at this time. A second step of the study is evaluating the effects of repetitive administration of the optimal biologic dose. Toxicity has included fever and shaking chills. Depending on the capability of anti-Leu 2a antibody to reduce circulating suppressor cells, a clinical trial evaluating anti-tumor effects may be carried out. This contract expired April, 1986.

FOX CHASE CANCER CENTER (NO1-CM9-7314)

This contract has had a long, complicated history. This has primarily been the result of the troubles of the manufacturer of the DT generator and its replacement DT tubes. Each of the first three DT tubes failed prematurely. The fourth tube was installed and commissioned for radiotherapy at the Fox Chase Cancer Center Hospital in February, 1985. This tube treated a small number of patients, then failed in early June. The source of the trouble was diagnosed, a solution identified and repair effected. It failed again in October before treating any more patients. Because the root cause of this long series of problems could not be corrected at reasonable cost, the contract has been terminated.

FRED HUTCHINSON CANCER RESEARCH CENTER (NO1-CM4-7668)

On this contract, a Phase I clinical trial is being conducted with the anti-melanoma monoclonal, MG21. This monoclonal antibody reacts with the sialo-ganglioside, GD_3, which is preferentially displayed on the cell surface of most melanoma cells. Pharmacokinetics, clinical toxicity, and immunological response

will be monitored. Once this trial is completed, a second Phase I study of MG21 and recombinant interleukin-2 will be initiated. This contract expired June, 1986.

GENE LABS, INC. (N43-CM5-7763) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this proposal is to develop an in vitro immunization system to produce human monoclonal antibodies to cell surface antigens of human breast carcinoma, colon carcinoma and malignant melanoma. Human monoclonal antibodies are desirable for a variety of reasons, including: (1) the majority of antibodies produced by rodents immunized with human cells are against species-specific rather than polymorphic determinants; (2) species-specific carbohydrate portions of immunoglobulins are important in several antibody effector functions; and (3) when murine antibodies are used therapeutically, it is likely that their activity will be neutralized by the formation of human anti-immune Ig. Tumor-sensitized B lymphocytes will be generated by this in vitro system and fused with a newly described human-mouse cell line, SBC-H20, that efficiently produces long-term stable human immunoglobulin secreting hybridomas. A detailed study of the in vitro immunization system will be performed in Phase I of this proposal to determine the optimum time for collection of B lymphocytes for fusion. Antibodies generated by this system will be characterized by reactivity to primary tumor and with other allogeneic tumor cells using frozen and paraffin sections, and other normal tissue controls.

GENE LABS, INC. (N43-CM5-7773) (SMALL BUSINESS INNOVATIVE RSEARCH Program)

Interleukin 2 (IL-2) is a relatively well studied lymphokine that promotes proliferation and differentiation of T lymphocytes and natural killer cells. IL-2 has potent anti-tumor effects when administered to experimental mice with metastatic disease. Unfortunately, initial efforts to treat humans with doses of IL-2 comparable to those used in the mouse led to unacceptable toxicity of types not predictable from the known actions of the lymphokine. We propose to produce mouse antibodies that bind to IL-2 receptors on lymphocytes and mimic IL-2 in their functional effects. Such antibodies should have a much longer half-life in vivo than IL-2 and should not necessarily produce the undesirable toxicity associated with high doses of IL-2. Specifically, we will produce neutralizing anti-IL-2 antibodies and then generate anti-idiotype antibodies against the neutralizing immunoglobulin. IL-2 mimetic antibodies will be identified on the basis of their ability to support the growth of IL-2 dependent lymphocytes.

GENERAL SOFTWARE CORPORATION (N43-CM5-7857) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this Phase I contract is to establish feasibility for a computerized pharmaceutical tracking system for monitoring the manufacture and quality of investigational products used in the Division of Cancer Treatment. This contract expired March, 1986.

GENZYME, INC. (N43-CM5-7787) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Heteroantisera against Interferon (IFN) and Interleukin-2 (IL-2) are highly immunosuppressive when injected into experimental animals. These anticytokines impair the generation of cytolytic T-cells, the rejection of transplanted tumors, and other cellular immune functions. Murine monoclonal antibodies against IL-2 have been available for several years, and recently the technology for the generation of human monoclonal antibodies has emerged. Patients treated with IFN in various clinical trials and patients with systemic lupus erythematosis with high endogenous levels of IFN develop antibodies against IFN. Similarly, patients repeatedly injected with recombinant IL-2 as treatment for refractory cancer develop IgG antibodies against IL-2. We propose to generate human hybridomas from the B-lymphocytes of these patients by fusion with human myeloma cell lines. The culture supernatants of these hybridomas will be screened for anti-IL-2 antibodies with a sensitive ELISA and subsequently for the ability to inhibit IL-2 induced T-cell proliferation, cytolytic T-cell induction, and the development of a delayed-type hypersensitivity response to tuberculin.

GEORGETOWN UNIVERSITY (NO1-CMO-7437)

On this contract Phase I and II clinical trials of lymphoblastoid and recombinant interferon-alpha are being conducted in patients with metastatic melanoma. In the first step of this study, three different schedules of lymphoblastoid interferon were compared in a total of 32 patients. Four patients have shown a complete or partial response. Responses were seen in both the alternate day and daily schedule, but not on the weekly schedule. The most significant toxicity was observed in one patient, who developed a lupus-like reaction while receiving the alpha interferon. This contract is now comparing the immunological effects of three different doses of recombinant alpha interferon in patients with melanoma. This contract expired April, 1986.

GEORGIA, UNIVERSITY OF (NO1-CM2-7401)

This contract, with the Department of Pharmaceutics of the University of Georgia, has the responsibility of performing shelf life evaluation of clinical drugs. This stability data is supplied to the Food and Drug Administration in support of the NCI's IND filings. This Contractor monitors the stability of dosage forms at several storage temperatures. The testing involves the use of multiple analytical methods. The method most frequently used for assay of the stability samples is high performance liquid chromatography (HPLC). This Contractor also has the responsibility of conducting reserve sample inspections as required by the U.S. FDA Current Good Manufacturing Practices.

GEORGIA INSTITUTE OF TECHNOLOGY (NO1-CM2-7517)

This project is one of the three contracts whose objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility; (b) increase compound stability; (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles. This contract is being recompeted.

GRUPE, I.S., INC. (N43-CM5-7814) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this Phase I contract was to establish feasibility for a computerized pharmaceutical tracking system for monitoring the manufacture and quality of investigational products used in the Division of Cancer Treatment. This contract expired March, 1986.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM2-3911)

This contract operates the Animal Production Area at the Frederick Cancer Research Facility (FCRF). The contract operates as a Primary Genetic Center, Rederivation Center and Embryo Freezing Center. Strains are received from the NIH Repository and rederived for use at the FCRF and distribution to other NCI contract activities. The bulk of the production on this contract is for supplying the animal needs of the researchers located at the FCRF. Animals are also sent from FCRF to other NCI funded research activities.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM3-7623)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for the many NCI research activities. This contract effort is currently being competed.

HARLAN/SPRAGUE-DAWLEY, INC. (NO1-CM5-7728)

This Rodent Production Center contract produces CD2F1 hybrid mice under maximum barrier conditions.

HAZELTON LABORATORIES AMERICA, INC. (NO1-CM4-7649)

This contract provides animal facilities to house rodents including mice, rats and rabbits. The animals are used for the study of tumor biology and immunology which is conducted by the Surgery Branch, NCI.

HIPPLE CANCER RESEARCH CORPORATION (N43-CM5-7829) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

In this Phase I contract, human tumor cell lines and cells from fresh tumor specimens were utilized to establish optimal culture conditions (cell) density, culture volume, growth medium, etc.) for growth of human tumor cell colonies in glass microcapillaries. In addition, a prototype instrument for scoring of colony formation was developed and evaluated. Results in this novel culture system compared favorably with a "conventional" two layer soft agar method.

HIPPLE CANCER RESEARCH CORPORATION (N43-CM5-7831) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this contract is to standardize in vitro human bone marrow progenitor assays for use in quantitative and qualitative measurements of the toxic effects of known anticancer drugs. These toxicities in bone marrow

culture are correlated with clinical hematotoxicity. In vitro studies of drug induced myelotoxicity have largely used mouse bone marrow. However, because of the differences in murine and human pharmacokinetics, drug metabolism and drug sensitivity of hematopoietic precursor cells, such murine studies often have not provided useful quantitative data on levels of clinical hematotoxicity in humans.

HOWARD UNIVERSITY (NO1-CM2-7543)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy. This contract terminated January, 1986.

HYBRITECH, INC. (NO1-CM6-7718)

This Contractor performs coupling of chemotherapeutic drugs, toxins and radio-isotopes to monoclonal antibodies directed against specific antigens found on human tumor cells. Appropriate tests are carried out on conjugates to demonstrate that the cytotoxic agent-antibody conjugates retain antigen-antibody specificity comparable to the unmodified antibody and cytotoxicity in excess of the nonderivatized cytotoxin. This Contractor is required to scale up the appropriate conjugation procedure to provide sufficient quantities of a human use product for preclinical and preliminary clinical trials. Experiments have been ongoing to couple methotrexate, ricin A chain, Yttrium-90, Indium-111 and Iodine-131 to T101, an antibody directed against a human T-cell differentiation antigen, and 9.2.27, an antibody directed against a human melanoma cell antigen. This Contractor has been supplying T101 conjugated to indium and iodine for biodistribution, imaging and therapy studies and is scaling up to provide T101 and 9.2.27 drug and toxin conjugates for clinical studies. This contract was awarded in September, 1985.

IDEC PARTNERS, INC. (N43-CM5-7766) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this application is to develop monoclonal antibody (MoAb) anti-idiotype (anti-Id) tumor vaccines for the treatment and prevention of cancer. The specific aims of the Phase I proposal are to define the general principles for maximizing the in vivo efficacy of MoAb anti-Id tumor vaccine preparations. This will be accomplished through a systematic comparison of anti-Id vaccines representing different types of tumor associated antigens (TAAs) in a solid tumor animal model. These Phase I studies will identify the critical variables affecting the therapeutic efficacy of anti-Id tumor vaccine preparations. In contrast to conventionally purified TAA vaccines, MoAb anti-Id preparations can be readily produced in large quantities and unlike recombinant DNA vaccines, anti-Id vaccines may be employed to generate immune responses against a wider variety of TAA determinants, including carbohydrate and lipid moieties.

IIT RESEARCH INSTITUTE (NO1-CM4-7567)

This contract, awarded as a result of the recompetition of the in vivo screening program, has a current level of testing in mice of potential anticancer agents of 18,000 L1210 equivalent tests per year, having been reduced during FY-85 due to budgetary restrictions. The contract provides for in vivo testing in the P388 leukemia pre-screen, for evaluation of materials in specified tumor panel models, for detailed evaluations requested by members of the NCI staff, and for characterizations and evaluations of tumor models as directed by the NCI Developmental Therapeutics Program. Materials tested in the P388 leukemia prescreen are new synthetic compounds and fractions of natural products provided by the NCI. Models of the conventional tumor panel now in use under this contract include the M5076 sarcoma and L1210 leukemia. Xenograft models include the breast subrenal capsule model, the LOX amelanotic melanoma, and the human lung A-549.

ILLINOIS, UNIVERSITY OF (NO1-CM6-7705)

The objective of this contract is to perform a survey of the literature published worldwide on natural products and related fields for the purpose of finding new and novel compounds that may have biological activity useful in the treatment of cancer. Reports of biological activity of extracts of plants, animals, bacteria, fungi, and marine organisms are also provided. This is a key contract for the acquisition of new agents for the DTP screening program. This contract has just been awarded for a three-year period (December 31, 1985 to December 30, 1988), and supersedes University of Illinois Contract NO1-CM3-7513, which expired December 30, 1985.

ILLINOIS CANCER COUNCIL (NO1-CM4-7667)

Under a Master Agreement, a Phase Ia trial is being conducted to evaluate the clinical toxicity and immunological effects of recombinant interleukin-2. Sixteen patients have been entered at each of four starting dose levels. Additional patients will be entered at higher dose levels, if dose-limiting toxicity is not observed at the highest protocol dose level to establish a maximally tolerated dose.

In addition, another trial is evaluating the clinical effects of a murine anti-T-cell monoclonal immunoconjugate, T101-I^{131}. Pharmacokinetics, relevant biologic responses, and clinical toxicity are being monitored. In addition, localization in tumor and normal tissues is being compared. To date, 16 patients have been studied, and no significant toxicity has been observed at the highest dose level. Additional patients will be entered at higher dose levels for further evaluation of dose, toxicity, and anti-tumor response.

In addition, another trial is being conducted to look for optimal biologic response of recombinant interleukin-2. Patients with Dukes' C colorectal cancer, who are at high risk for relapse, will be given bi-weekly 24-hour infusions of interleukin-2 for an extended period of time. Extensive clinical and immunologic monitoring will also be performed. This trial began in early Spring.

IMMUNOMEDICS, INC. (N43-CM5-7778) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The continuing development and evaluation of new radiochelation agents is of utmost importance in tumor imaging with anticancer antibodies. While DTPA-labeled In-111-radioimmunopharmaceuticals are widely used as tumor imaging agents, their high liver background is a disadvantage in providing a high target-to-background ratio. The proposed project envisages the design and synthesis of specific organic compounds which are expected to be more lipophilic than DTPA and known to complex radiometals in order to develop a better chelate for labeling antibodies with metals. Efforts will be directed toward the synthesis of new compounds, bisthiosemicarbazones 4 followed by purification and characterization of the compounds by chemical, chromatographic and spectroscopic techniques, and elemental analysis. The next stage is directed toward the antibody conjugation of chelating agents, radiolabeling with Tc-99m and separation of the desired product. This will be followed by studies of the in vitro stability of the complexes to evaluate the suitability of the chelating agent for use in the radioimmunodetection of cancer.

IMMUNOMEDICS, INC. (N43-CM5-7786) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This project will evaluate antibody mediated, polymer bound Yttrium-90 as a cancer therapy agent. Antibodies will be coupled through one covalent bond to a small polylysine unit. The latter will carry, via a bifunctional coupling agent, molecules of the trivalent metal chelating siderophore, deferoxamine. Each deferoxamine will be complexed with a single atom of Yttrium-90, a high energy beta-emitter. The optimum number of deferoxamines, bindable to the epsilon amino groups blocked to ensure non-interference in antibody conjugation. The polylysine-deferoxamine linker used in this laboratory has been shown to work very well in similar systems, and will be applied here. In the scheme we propose, we have two distinct routes for coupling polymer to antibody, both based on well documented methodology.

IMMUNOMEDICS, INC. (N43-CM5-7851) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Colon-specific antigen-p(SCAp) is a comparatively new, organ specific, oncofetal antigen for colorectal cancer. The object of this project is to produce a kit that utilizes anti-SCAs monolonal antibodies (MoAb's) for clinical radioimmunodetection (RAID) of colorectal and pancreatic cancers. Phase I will be devoted to selection of hybridomas with specificity to SCAs, and MoAb's that are suitable for imaging. It will be achieved by investigating their in vivo distribution in tumor and nontumor tissues using an animal model.

IMMUNOTECHNOLOGY OF INDIANA (N43-CM5-7785) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

We propose to develop a standardized kit of monoclonal antibody (MoAb) conjugated with a novel biological catalyst toxin to be used for the ex vivo elimination of neoplastic cells from human autologous bone marrow grafts. The technical objectives are to prepare a toxin-conjugated murine MoAb that will specifically bind to antigen positive neoplastic cells present in autologous bone marrow grafts but not to antigen negative cells. This toxin conjugated MoAb will efficiently lyse the antigen positive cells to which it is bound without substantially affecting antigen negative stem cells necessary for restoration of hematopoietic and immunologic reconstitution of the host. The methods outlined in this proposal are

based on a class of catalyst-toxins that have not previously been considered for application to ex vivo cytolysis of neoplastic cells. These toxins have been extensively studied and shown to mediate highly efficient lysis of neoplastic cells. Moreover, it has been established that these catalysts can be conjugated to MoAb with retention of antibody specificity and toxin activity.

INET CORPORATION (N43-CM5-7860) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A total of five SBIR contracts covering two topics in Phase I have been managed by the Information Technology Branch, DTP, during the reporting period. This contract calls for the design of a new version of the Drug Information System that would run on a 32-bit computer.

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CM2-7510)

This contract supports the information needs of the Cancer Therapy Evaluation Program by providing comprehensive information management during the protocol review process, providing data on the objectives of both active and completed protocols, and providing data on the results of active and completed protocols. The system provides scientific and administrative information on: 1) treatment modalities (e.g. drugs, biological response modifiers, radiation, and surgery); 2) diseases; 3) protocols; and 4) investigator teams.

In addition, a subcontracted effort to VSE Corporation provides for the maintenance and operation of the Drug Distribution and Protocol Monitoring System (DDPMS). The DDPMS is an automated procedure used to verify the accuracy of investigator drug requests, thus fulfilling our legal (FDA) requirements in that regard. Verified data is retained and forms a drug distribution history which is used to monitor protocol activity as clinical trials progress. The system also provides management information for the program, the cooperative study group and private organizations. The project is being recompeted.

INSTITUT JULES BORDET (NO1-CM5-7645)

Materials collected in Western Europe are screened in vivo against animal tumors in accordance with established NCI protocols. Materials that originated in the U.S. or other countries may be sent to this laboratory for testing. Testing is currently being conducted at a level of approximately 10,000 L1210 test equivalents per year. More detailed evaluation of drugs of interest to NCI is conducted upon request or agreement of the Project Officer.

INTERMAGNETICS GENERAL CORPORATION (N43-CM5-7801) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Two working demonstration NMR (nuclear magnetic resonance) microscopes have been developed, and several images of biological samples such as water phantoms, celery, and fruit fly pupae have been obtained at a magnetic field of 2 Tesla with spatial resolution approaching 10 microns and correlated with optical and electron microscopy. In vivo proton magnetic resonance spectra have also been obtained in the fruit fly experiments. Special ultraminiature rf (radiofrequency) coils and sample holders have been designed and tested to permit NMR microscopy at both 2 Tesla and 14.2 Tesla. (The latter work has been done in collaboration with Carnegie Mellon Institute, where the 14.2 Tesla magnet is the highest NMR field system presently available in the world). Future improvements are aimed

at development of instrumentation which can image and characterize cancer cells and tumors microscopically from the standpoint of their magnetic properties.

IOWA, UNIVERSITY OF (NO1-CM3-7561)

This contract provides services involving dosage form development and manufacture of investigational drugs for subsequent clinical evaluation. Compounds to be formulated are selected and provided by the National Cancer Institute. This Contractor has primarily developed and produced sterile freeze-dried injectable products under this contract. However, this contractor has the capability to produce a wide variety of pharmaceutical dosage forms. This contractor is also responsible for completing all required quality control tests on each lot of drug. All products are packaged, labeled, and shipped to the National Cancer Institute for redistribution to clinical investigators. This contract is being recompeted.

IOWA, UNIVERSITY OF (NO1-CM3-7579)

This contract provides capabilities to chemically characterize peptides, proteins and glycoproteins that may be used experimentally and/or clinically to modify tumor growth. Assay methods are developed to analyze the substance in bulk dosage form and in common pharmaceutical vehicles. Studies include determination of amino acid composition, molecular weight, isoelectric point, terminal sequence and development of suitable immunological measurement (radioimmunoassays, etc.) and suitable biological assays for qualitative and quantitative evaluations. In the past year this contractor has analyzed and characterized several lots of naturally occurring alpha, beta and gamma interferons for activity and purity, analyzed several natural and recombinant IL-2 preparations, natural B-cell growth factor preparations, natural human beta interferons, human tumor necrosis factor, human thymic factors, human and murine colony stimulating factors, and purified the tetrapeptide tuftsin for preclinical screening. This Contractor has also examined a murine tumor necrosis factor preparation for purity and provided near homogeneous material for monoclonal antibody production. This contract expired September, 1986, and will be recompeted.

IOWA, UNIVERSITY OF (NO1-CM4-7594)

This resource contract provides the Division of Cancer Treatment with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms are manufactured in conformity to U.S. FDA Current Good Manufacturing Practices and in a manner to protect personnel from exposure to potentially toxic agents. This Contractor is also responsible for all required quality control tests on each product prepared. These dosage forms are packaged, labeled, and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CMO-7338)

A major effort in breast cancer has been through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have set a standard for therapeutic studies in this country and around the world. The Istituto has shown an improved overall survival for premenopausal patients treated with CMF, and that 12 months of CMF is no more effective than six months. More recent analyses indicate that survival of patients following relapse is

unaffected by whether or not they received adjuvant chemotherapy, and that estrogen receptor status is an independent prognostic indicator for pre-menopausal women. Istituto has developed an extensive data base on the utility of labelling index, which appears to be a more powerful prognosticator than hormone receptor status. Patient accrual will continue through September, 1986.

JAPANESE FOUNDATION FOR CANCER RESEARCH (NO1-CM3-6011)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo to provide up-to-date information services in support of our cancer treatment program, both preclinical and clinical. This is a cost-sharing contract and is strongly supported by the Japanese Foundation for Cancer Research and the Japan Society for the Promotion of Science.

JEFFERIES ASSOCIATES (N43-CM5-7795) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Jefferies Associates wishes to apply its proprietary stabilization technology to the problem of rapid liposome uptake by the RES. We believe, that with proper surface modification of the liposome microcarrier, utilizing surface binding of a specific membrane-bound glycoprotein, the circulatory half-life of injectable liposomes can be increased significantly. This hypothesis will be tested by *in vitro* macrophage uptake of altered liposomes and a direct comparison to control phospholipid vesicles.

JOHNS HOPKINS UNIVERSITY (NO1-CM5-7738)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with Spiromustine, Taxol, Trimetrexate, HMBA, and N-Methylformamide (NMF).

KANSAS, UNIVERSITY OF (NO1-CM3-7562)

This contract investigates approaches to resolve difficult dosage form development problems not amenable to usual solubilization or stabilization methods. This Contractor has considerable expertise in the application of molecular complexes and reversible derivatives to improve solubility. This Contractor also is responsible for pilot scale preparation and chemical analysis of the formulations developed under this contract. This contract is being recompeted.

KARYON TECHNOLOGY, INC. (N43-CM5-7828) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this contract was to evaluate the ability of GELTRAP, a matrix of calcium alginate gels, to grow human tumor cells and fresh human tumor specimens in culture. The system allowed for the handling, feeding and growth of cultures in the presence or absence of anticancer agents, and the gels could be resolubilized by chelation with EDTA or similar agents for recovery of the entrapped cells for quantitation or other purposes. This contract expired March, 1986.

KENTUCKY RESEARCH FOUNDATION, UNIVERSITY OF (NO1-CM3-7620)

Difficult dosage form development projects not amenable to the usual solubilization and/or stabilization approaches are assigned to this contractor. This Contractor has particular expertise in the application of reversible derivatives (prodrugs) to improve drug solubility. Pilot batch preparation and chemical analysis of these novel formulations are carried out under this contract. This contract is being recompeted.

KESA CORPORATION (N43-CM5-7808) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Extensive research on reduction of "speckle" artifact in diagnostic ultrasound images has led to increased detectability of low contrast images using frequency diversity techniques. Use of the new techniques in controlled experiments has demonstrated the ability to detect objects in the processed images which were virtually unidentifiable in the unprocessed images. The processing gives these objects well defined boundaries and improved subtle differences in ultrasound reflection characteristics. All of these enhancements are useful in diagnostic ultrasound examinations in increasing sensitivity and the ability to characterize diseased states of tissues noninvasively.

KOBA ASSOCIATES (NO1-CM4-7661)

This contract provides technical support for the collection, review and compilation of available information on biological response modifiers for possible development through preclinical and clinical trials. The Contractor has responsibility for obtaining pertinent information from sources in industry, institutes, universities as well as through literature review. Information and references obtained are submitted to the program in the form of review articles and one page synopses. Information gathered is stored in a computer data bank. In the final period the contract will provide about five synopses and reviews and update all preclinical and clinical files. This contract will expire January, 1987.

LASERMED CORPORATION (N43-CM5-7809) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A laser-based system has been designed and is being developed for clinical diagnosis and treatment of lung cancer. The diagnostic portion of the system employs a krypton ion laser which emits light in the range of wavelengths from 406-416 nanometers through a fiber optic system. Premalignant and malignant cells which have absorbed hematoporphyrin dye derivative will fluoresce under this light and can be detected and visualized. Sensitivity is increased by computer-based image enhancement. If these malignant cells are then illuminated through the system by red light from a different laser at 630 nanometers, they can be destroyed.

LASERSCOPE (N43-CM5-7833) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this SBIR contract is to develop a coupled laser system for photodynamic therapy and to test the system on cellular, animal models, and possibly human systems. The company's laser surgical system (YAG/532nm) will be coupled to a dye laser to produce a stable red laser beam of 2-3 watts of 625-630 nm. The appropriate fiber optic and delivery systems for the dye laser will also be developed.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CM3-7625)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for the many NCI research activities. This contract terminated July, 1986 and will not be renewed.

LITTON BIONETICS, INC. (NO1-CMO-5724)

This contract provides animal facilities to house small and large animals, including mice, rats, dogs, rabbits, goats, and monkeys. The animals are used for the preparation of antibodies as well as for inoculation of tumor cells and and virus preparations for tumorigenicity testing.

LITTON BIONETICS, INC. (NO1-CM2-5616)

The major objectives of this contract are: (1) to purify and supply factors that promote growth and differentiation of myelogenous leukemic cells and T cells; (2) to purify the envelope and internal structural proteins of type C RNA tumor viruses; (3) to prepare monoclonal antibodies against the purified structural proteins; (4) to provide cultured T and B cells from human cord blood, peripheral blood, and leukemic cells; and (5) to prepare and supply radiolabeled cDNA and RNA probes from type C retroviruses.

LITTON BIONETICS, INC. (NO1-CM3-7575)

The major objectives of this contract are: (1) the use of immunofluorescence and radioimmunoassays to screen human T cells and T cell lines for human retroviral structural protein expression; (2) the use of ELISA assays to detect antibodies against human retroviruses in serum from leukemic patients, AIDS and ARC patients, and normal donors; (3) to test culture fluids from short-term and long-term cultured cells for the presence of viral DNA polymerase activity; and (4) to test sera from patients with T cell leukemia, AIDS, or ARC for antibodies to human type C RNA tumor virus (HTLV-I, -II, and -III).

LITTON BIONETICS, INC. (NO1-CM3-7582)

This contract provides supportive services in the supply of small quantities of T and B cells grown in tissue culture, partially purified IL-2, and radiolabeled cells and nucleic acids.

LITTON BIONETICS, INC. (NO1-CM4-7651)

The major objectives of this contract are to prepare and supply large quantities of concentrated and purified human type C RNA tumor viruses (HTLV).

LUNAR RADIATION CORPORATION (N43-CM5-7799) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The Lunar Radiation Corporation intends to construct a detector array that will be mechanically scanned in conjunction with a scanning fore-slit at the exit of

the x-ray tube. The fore-slit will be integrated into the DBA printer housing. Specifically, they plan to conduct a study of possible detector materials from a performance and economics point of view, to determine a suitable detector geometry and scanning configuration including fore-slit mechanism, to design and build interfaces to the new University of Wisconsin (ISC) DBA printer and x-ray generator, and to test the performance of the detector using the Humanoid anthropomorphic phantom.

LUXTRON CORPORATION (N43-CM5-7841) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this Phase I effort is to design, fabricate, and evaluate prototype catheter temperature sensors that meet the requirements for interstitial implantation, microwave antenna insertion, and temperature monitoring for feedback and control during hyperthermia treatments.

MARYLAND, UNIVERSITY OF (NO1-CM5-7734)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment, Phase II/III studies in patients with disseminated solid tumors and leukemia, or Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with CBDCA, HMBA, and Menogaril. Phase II/III studies include Methotrexate + CBDCA in patients with head and neck cancers; CBDCA + radiation therapy for unresectable head and neck cancers; DxDx in non-small cell lung cancer; NMF in colorectal carcinoma; NMF in renal carcinoma; CBDCA in renal carcinoma; CBDCA in gyn malignancies; high dose BCNU/ L-PAM/Vincristine in malignant melanoma; AZQ in acute leukemia, and Caracemide in advanced or recurrent colorectal cancer.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM1-7481)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM2-7532)

This Contractor contributes proton data to a collaborative effort to improve treatment planning with heavy particles, to include protons, helium and heavy ions, and neutrons. In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, each contractor contributes patient data to other contractors that are members of the collaborative working group. Treatment plans developed by each contractor using the same patient data will be compared using evaluation methods developed by the group. A table of normal tissue tolerances for various organs has been developed by the working group.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM4-7616)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CM4-7687)

This is one of five contracts awarded in May, 1984 (1) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) systems in the detection and diagnosis of pathology in human subjects and in the non-invasive characterization of tissues, and (2) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with that from other modalities (e.g., CT and radioisotope imaging). This Contractor, working as a member of the MRI Collaborative Working Group (CWG), has contributed to the development of clinical imaging protocols for evaluation of MRI in the diagnosis of musculoskeletal tumors, cervical myelopathies, uterine neoplasms, liver metastases, brain neoplasms, and congenital heart disease. They have also provided coordination for the CWG of the development of the musculoskeletal tumor and liver metastases protocols. Patient imaging has begun in each of these studies. This Contractor has been influential in guiding the statistical design of several of the protocols.

MAXIMA CORPORATION (NO1-CM4-7628)

The objective of this small business contract is to perform a variety of computer searches such as full structure searches, substructure searches and data item searches in support of the DTP program. This Contractor utilizes several data bases such as DIS, DARC, Questel, NLM, and Dialog. Another task under this contract is the development of chemical names for compounds of interest.

MAYO FOUNDATION (NO1-CM2-7528)

This contract is part of a collaborative effort among three institutions to investigate the role of intraoperative radiotherapy in the treatment of intra-abdominal malignancies and to develop guidelines for intraoperative radiotherapy techniques for use by other investigators and by clinical cooperative groups in Phase II and III clinical trials. An optional task is to investigate the use of radiation modifiers in conjunction with intraoperative radiotherapy. The collaborative effort will facilitate the evaluation of this new radiotherapeutic modality with respect to normal tissue tolerance, safety, and efficacy.

MAYO FOUNDATION (NO1-CM3-7601)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor studied or is studying three drugs under three separate task order contracts: Teroxirone (NSC 296934), Anthrapyrazole (NSC 349174) and Diazohydroxide (NSC 361456). This contract was conducted in three phases:

Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; and Phase III, infusion dose pharmacokinetics. The information will be used where appropriate for IND filing and by Phase I investigators in determining appropriate schedules for administration. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

MAYO FOUNDATION (NO1-CM5-7711)

This is one of three contracts devoted to the application of a human tumor colony forming assay (HTCFA) to drug screening. Contract efforts have focused on screening compounds inactive in the standard in vivo pre-screen (murine P388 leukemia) to establish the potential of the HTCFA to identify compounds not detected by the in vivo screen. Materials active against fresh human tumor specimens are also tested in a P388 cell line colony forming assay, developed under this project for direct comparison with the HTCFA. Screening has been initiated on more than 300 compounds and several have been identified as antitumor drug leads. Activity in the HTCFA, P388 colony forming assay, in vivo tumor panel, and degree of structural novelty, are used to determine which are recommended to the Decision Network Committee for development to clinical trial. To date three compounds have been accepted into the formal drug development program.

MAYO FOUNDATION (NO1-CM5-7733)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment, Phase II/III studies in patients with disseminated solid tumors and leukemia, or Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with Hexamethylmelamine, 6-Thioguanine, Pibenzimol, Nafidimide, Recombinant Interferon αA plus BCNU, and recombinant human tumor necrosis factor (TNF). A Phase II study is presently being conducted with Menogaril in patients with renal cancer.

MAYO FOUNDATION (NO1-CM6-7904)

Preclinical pharmacology studies of antitumor agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data as well as the efficiency of the Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to preclinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of antitumor agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because of the increasing emphasis on in vitro screens which is contemplated in the next several years. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed pre-

clinically, the Phase I clinician is alerted to their potential human significance. This is a new Task Order Managed contract and replaces the previous Pharmacology Master Agreement.

MEDALEASE, INC. (N43-CM5-7852) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Despite improvement in the control of local disease, development of metastases, especially pulmonic metastases, continues to be a challenging therapeutic problem in sarcoma patients. Our hypothesis is that radiolabeled antisarcoma monoclonal antibody may aid or improve the detection of small or occult sarcomatous deposits in these patients. We have recently produced a mouse monoclonal antibody (Mab 19-24) which is broadly cross-reactive with human sarcomas, and have administered it to animals and patients without significant toxic effects. Using imaging techniques we have detected tumor deposits in animals and patients who received the radiolabeled antibody. The smallest human tumor detected measured 1.5 x 1.0 x 0.5 cm. Most recently we have detected right lower lung metastases in two patients. Diagnosis was made exclusively by antibody scan. Futhermore, gamma counter analysis of operative specimens has consistently shown increased radioactivity in tumor deposits.

The specific aims of this research are: 1) to determine optimal conditions for sarcoma imaging in an animal system and in patients using monoclonal antibody 19-24 labeled with ^{125}I, ^{131}I and ^{111}In and 2) to use the selected optimal form of antibody 19-24 and radiolabel to determine if small tumor foci (e.g., 1-2 cm pulmonic metastases) in sarcoma patients can be successfully imaged.

MEDICAL FACULTY ASSOCIATES (N43-CM5-7769) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A human T-cell lymphokine capable of interrupting the proliferation of myeloid leukemia cells and inducing terminal differentiation to monocyte-macrophage has been described. This "maturation inducer" (or differentiation factor) is produced by normal T helper cells and has been shown to be a T cell mediator of monocytic cell development. This application is aimed at the production of this lymphokine for development and evaluation as an immunoaugmentive agent with potential antileukemic activity. Rat monoclonal antibodies that have already been shown to bind the inducer activity will be employed to construct immunoabsorbant columns which will be used with medium from HUT cells and normal lymphocyte conditioned medium to purify the inducer activity. The yield and specific activity of the inducer thus obtained will be determined along with an evaluation of the biological activity. This includes analysis of HL-60 cell differentiation to mature monocytic cells as judged by cellular morphology, differentiation antigens, expression of membrane complement receptors, phagocytosis, etc. Simultaneous analysis of cellular proliferation will be investigated by cell cycle phase kinetics with flow cytometry. The purity of the inducer obtained will be evaluated by gel electrophoresis, column chromatography and HPLC.

MEDICAL LASER RESEARCH AND DEVELOPMENT CORPORATION (N43-CM5-7832) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor will show the feasibility of developing an instrument that delivers two different laser beams to the same site: one beam to be optimized for cutting without adjacent tissue damage and the other to be optimized for hemostasis. The company will use a rapid sweep dye laser to control hemostasis

during mechanical (steel scalpel) incision with the hemostasis laser in the same manner that it would be used in combination with the cutting laser. The morphological changes produced by the laser induced hemostasis will then be characterized by histologic examination.

MELOY LABORATORIES, INC. (NO1-CM4-7650)

The purpose of this contract is to provide effective inventory, distribution and quality assurance confirmation for biological response modifiers. The Contractor is responsible for receipt, dispensing, storage, distribution and inventory control of biological agents. Quality assurance evaluation involves specific assays to confirm sterility and assays to determine pyrogenicity and endotoxin levels. This Contractor performs general safety tests for biological agents in compliance with Government regulations intended for clinical use and helps in the development of master files and IND's for biologics. Currently, this Contractor provides for storage and distribution of approximately 100 different biologics. In the past year the Contractor has performed general safety, pyrogenicity, purity and other relevant testing on several lots of monoclonal antibody preparaations for use in clinical trials from within the program, as well as preparations submitted from other NCI and NIH scientists. The contract also provides for ascites production and purification of monoclonal antibody and has produced monoclonals specific for melanoma, colon, breast cancers, T-cell receptor, and lymphocyte antigens. This contract will be recompeted in FY 1987.

MELOY LABORATORIES, INC. (NO1-CM5-7688)

This contract supports Surgery Branch research by providing appropriate amounts of human and murine Interleukin-2 to conduct experiments. In addition, murine and human lymphokine activated killer cells are generated for therapeutic experiments. This research is directed toward developing new adoptive immunotherapies for the treatment of cancer using specifically sensitized lymphoid cells expanded in Interleukin-2 or using Interleukin-2 directly as an immune adjuvant.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM4-7695)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-ofthe-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7732)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment, Phase II/III studies in patients with disseminated solid tumors and leukemia, or Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with MMPR/PALA/FU, PALA/MTX/LEU/FU, CBDCA/DVA/ BLEO, Trimetrexate, and HMBA. Phase II/III studies include Rand. Good Risk - GCT, CBDCA - lymphoma, Gallium Nitrate - prostate, Fludarabine - lymphoma, HHT + VP-16 - lymphoma,

CBDCA - GCT, CBDCA - melanoma, NMF - melanoma, CBDCA - osteosarcoma, Echinomycin - cervix, Menogaril - pancreas, CBDCA/VP-16/Bleo, Ifosphamide-Mesna - esoph, and Ifosphamide-Mesna - colorectal cancer.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CM5-7776)

The objective of this collaborative effort is to develop recommendations and guidelines for a program in Interstitial Radiotherapy. The recommendations shall include calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and afterloading procedures where appropriate.

MEMORIAL SLOAN-KETTERING CANCER CENTER (NO1-CM4-7665)

This Contractor will evaluate the effects of recombinant interleukin-2 on the biologic efficacy of the anti-melanoma monoclonal antibody, R24 in patients with advanced disease. This monoclonal antibody is directed against the sialoganglioside, GD_3, which is preferentially displayed on the surface of most malignant melanoma cells. A dose of 10^6 units/m^2/day interleukin-2 will be used, while the dose of monoclonal antibody will be varied. This trial began in early spring, 1986. The contract expired December, 1986.

MIAMI, UNIVERSITY OF (NO1-CM6-7877)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility or a contract research laboratory. All testing is scheduled by the Project Officer. This contract replaced contract NO1-CM8-7230 which terminated December, 1985. Contract NO1-CM8-7230 was listed as Papanicolaou Cancer Research Institute, which is now a part of the University of Miami.

MICHIGAN, UNIVERSITY OF (NO1-CMO-7405)

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 200 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the NCI and the Principal Investigator. Patient accrual continued through July, 1985 and follow-up ended in February, 1986.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (NO1-CM4-7593)

The major objective of this contract is the isolation of new antitumor agents from fermentations of marine and terrestrial microorganisms. These fermentations are screened against various enzymatic and other biochemical screens. Active products are isolated in sufficient quantities to be evaluated at the

National Cancer Institute. In addition, various immunogen tests have been developed to evaluate the organisms and their metabolites as potential immunological stimulators specific for cancers. Two chemotherapeutic agents from this contract, aclacinomycin and deoxyspergualin, are in clinical trials under NCI sponsorship. This contract ended June, 1986.

MICROBIOLOGICAL ASSOCIATES (NO1-CM9-7287)

This contract functions in four major areas: 1) to operate and maintain a virus serum diagnostic laboratory. Serum samples are submitted from contract animal suppliers and testing laboratories; 2) to test experimental tumors (animal and human) for viral contaminants; 3) to perform ELISA tests annually for the detection of mouse hepatitis virus (MHV); and 4) to produce vaccinia virus which is used for immunizing mice against infectious ectromelia.

MICROWAVE MEDICAL SYSTEMS INC. (N43-CM5-7821) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Phase I SBIR contract examined the feasibility of using passive microwave radiometry to detect extravasation of intravenous fluids. The use of radiowaves to detect temperature differential between intravascular and extravascular tissues during infusion of fluid is the basis for this approach. Infusion fluid temperature and flow rate affected the response, but the cannula diameter did not. This contract expired March, 1986.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-7555)

Midwest Research Institute is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological, and clinical phases of the Developmental Therapeutics Program. This Contractor determines the identity and purity of the compounds by appropriate methods. This Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (paper, thin layer, gas liquid and high pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry, and other methods as needed. Reports of the work performed by the contractor provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

MIDWEST RESEARCH INSTITUTE (NO1-CM3-7604)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this contractor studied Melphalan (NSC 8806). The contract was conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. The information is to be used in a retrospective analysis in a Clinical Oncology Program study relating blood concentrations in mice and man to dose escalation schemes. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

MISSOURI, UNIVERSITY OF (NO1-CM2-7534)

This contract monitors the animal production and screening laboratory by testing for the presence of Salmonella and Pseudomonas. Samples are received on a scheduled basis from the animal producers and screening laboratories and approximately 9,000 fecal samples are tested per year.

MISSOURI, UNIVERSITY OF (NO1-CM6-7723)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. Special testing is provided on a priority basis when a suspected animal disease problem occurs at an animal supplier facility in a contract research facility. All testing is scheduled by the Project Officer. This contract replaced contract NO1-CM8-7157 which terminated December, 1985.

MONOCLONAL SERVICES (N43-CM5-7782) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Transforming growth factors (TGFs) can induce and maintain the transformed phenotype. TGFs are produced by tumor cells and can act on those tumor cells (autocrine effect) or can induce a reversible "transformed" state in normal cells, for example in normal rat kidney (NRK) cells grown in soft agar. TGFs have recently been purified and have been classified as alpha-TGFs, meaning epidermal growth factor-like, or beta-TGFs, which require EGF for function. Monoclonal antibodies (MoAbs) raised against TGFs may compete with TGF receptors for the binding of TGFs and may inhibit the action of TGFs on tumor or normal cells. This proposal describes the production of human MoAbs to alpha and beta TGFs using an in vitro immunization protocol using defined, serum-free medium. Purified TGFs will serve as antigen using human peripheral blood B cells. Mouse-human and human-human hybridomas will be constructed. Screening of MoAbs thus formed will be done by the inhibition of growth of NRK cell in soft agar.

MONSANTO RESEARCH CORPORATION (NO1-CM2-7516)

This service preparative contract provided for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared were not readily available on the open market or from the original supplier in the amounts and/or quality required. The effort of this contract was devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities. This contract expired March, 1986.

MORAVEK BIOCHEMICALS, INC. (N43-CM5-7830) (SMALL SUBINESS INNOVATIVE RESEARCH Program)

The objective of this contract is to synthesize radioactively labeled chemicals and drugs for the use in preclinical pharmacology, mechanism and drug metabolism studies. The majority of the material prepared are not available from commercial sources. All materials are analyzed for chemical and radiochemical purity. This contract provides storage facility for the labeled materials and distributes them as instructed by the National Cancer Institute.

N. MULLEN ASSOCIATES (N43-CM5-7861) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A total of five SBIR contracts covering two topics in Phase I have been managed by the Information Technology Branch, DTP, during the reporting period. This contract calls for the design of a new version of the Drug Information System that would run on a 32-bit computer.

NATIONAL ACADEMY OF SCIENCES (NO1-CM5-7644)

This contract Task Order serves to develop standards for animal care and maintenance; shipping standards for the various species of laboratory animals, standards for nomenclature used to identify stocks and strains of laboratory animals; standards for animal maintenance in the research laboratory; and laboratory animal procurement standards. These standards are formulated by ad hoc commmittees whose memberships represent commercial animal production colonies, governmental and academic instituions, and non-profit research institutions.

NATIONAL BUREAU OF STANDARDS (YO1-CM6-0129)

The aim of this contract is to improve the accuracy and consistency of measurements of absorbed dose for neutron radiation therapy by providing national dosimetry standards and improved data on neutron interactions with tissue and tissue-equivalent materials. This work will provide national standards for neutron dosimetry and facilities at the NBS for the calibration of instruments used to measure the dose delivered to neutron therapy patients.

NEORX CORPORATION (NO1-CM5-7719)

The purpose of this contract is to develop a centralized, coordinated program for uniform preclinical testing and evaluation of monoclonal antibodies (MoAb) and their immunoconjugates prior to entry into clinical trials. This Contractor will test and evaluate MoAb and immunoconjugates in several test systems: 1) immunoreactivity against a panel of known tumor cells to define relationships with other MoAb and establish epitope reactivities by molecular or serologic means; 2) in vitro cytotoxicity assays; 3) virus testing for LCM, retrovirus and the MAP test; 4) immunohistologic screening to define antigen positive tissues and specificity; 5) antitumor effects in the nude mouse model and subrenal capsule assay; and 6) animal toxicity evaluation in rodents and perhaps primates. MoAb will be evaluated at each level of testing and must exceed preestablished standards of specificity and activity before proceeding to the next level of evaluation. MoAbs successfully progressing through the screening process will then be considered for clinical evaluation. This contract was awarded in FY 1986.

NEORX CORPORATION (N43-CM5-7759) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this contract proposal is to develop new radioiodine labeled conjugates for monoclonal antibodies that can be used in diagnosis and therapy of cancer. The goal of our proposed research is to investigate a novel method of introducing high specific activity radioiodine into some small molecules that can be conjugated to monoclonal antibodies. Our approach to develop new conjugates is patterned after the commercially available Bolton-Hunter and Wood's reagents, where the radioiodinations are carried out in a separate reaction vessel so that the antibody is not exposed to the harsh oxidizing conditions of the radioiodination. One important difference between the mentioned reagents and the reagents

that we will be radioiodinating in our proposed studies is that the radioiodine will be attached to a non-activated aromatic ring. This should impart an increased stability against chemical and enzymatic degradation. This phase I study will include the synthesis of targeted compounds, studies of radioiodination reaction conditions, and studies leading to eventual radiopharmaceutical 'kit' formulations.

NEORX CORPORATION (N43-CM5-7760) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this Phase I SBIR project is to develop a Re-186 agent suitable for labeling bifunctional chelating agent antibody conjugates. Although chemically similar to Tc-99m, application of Re-186 to yield potential therapeutic radiopharmaceuticals presents chemical problems which have yet to be solved. In this project we will develop several procedures needed for the labeling of protein conjugates with Re-186. These will involve reduction of perrhenate using agents such as Sn(II), as well as ligand exchange procedures using Re-186 complexes such as $trReO_2(pyridine)_4^+$ and re-gluconate. Initial experiements will be conducted with the free bifunctional ligand, and subsequent experiments will utilize the antibody conjugates which are already available. Labeling yields will be monitored by HPLC techniques. In vitro immunoreactivity assessment will be done to assess the effects of radiolabeling and conjugation on antibodies. The ability to label antibodies with Re-186 is the first step towards developing a Re-186 radioimmunotherapeutic agent.

NEORX CORPORATION (N43-CM5-7780) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this project is to develop and characterize monoclonal antibodies which can be used in the form of radionuclide immunoconjugates for in vivo imaging of B-cell lymphomas. The Phase I project will be comprised of four components; production of the antibodies, in vitro characterization of antibody selectivity patterns, delineation of the extent to which various tumor cells are capable of binding the antibodies, and in vivo animal studies of tumor cell localization and biodistribution. The latter phase would employ NeoRx's proprietary technology for the chemical coupling to antibodies of technetium-99m, the most widely employed radioisotope in nuclear medicine.

NORINA INSTRUMENT COMPANY (N43-CM5-7803) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A color imaging display system has been developed in prototype form which permits the digitizing of standard recorded medical images from anatomical imaging systems (e.g., X-ray CT, ultrasound, MRI) or from electron or optical microscopy. Realtime processing of this information permits transmission and display of images with great enhancement of detail and in a scale of perceived image intensities that is both natural to the human observer and that corrects for the nonlinear psychovisual perception characteristics of the human observer. Research within this program has permitted the electronic simulation of expected eye perceptivities, thereby obviating the need for repeating perception tests by human observers on all new images. The use of the linearized spectrum and a pseudocolor display scale (ranging from black, red, and orange through yellow to white) has increased the ability to discern "just noticeable differences" (jnd's) in intensity by 30% over the usual gray scale. Electron microscope images that are barely discernible are seen in excellent detail using this new digital color imaging display system.

NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CM3-7578)

The capability for evaluating chemical compounds for radiation sensitizing and/or radiation protective properties is provided by this resource. Various physical-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radiosensitizing or radioprotective characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiosensitization using mammalian cell cultures. Potential radiosensitizing compounds which appear to be superior to the standard - misonidazole - will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radiosensitizers), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All radioprotective compounds tested will be compared with the standard - WR-2721. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds. This contract should provide new radiosensitizers and radioprotectors or leads in developing new types (classes) of radiation modifying compounds.

NORTHWESTERN UNIVERSITY (NO1-CM3-7538)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. This Contractor carries out the service by performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

NUCLEAR ONCOLOGY SERVICES CORPORATION (N43-CM5-7845) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

At the present time there is little quantitative information on the development of radiation-induced fibrosis after single or fractionated doses of radiotherapy. Recently, methods have been developed to assay fibrotic changes in tissue. To test the feasibility of using these assays to measure fractionation-induced fibrosis, animals are irradiated under appropriate conditions. Four months after exposure the synthesis of collagen in skin and lung is measured by the incorporation of 3H-proline into OH-proline (an amino acid virtually unique to collagen). Total collagen content is then measured in fixed, paraffin-embedded tissues from the same animals. The results of thse studies should demonstrate the feasibility of using the assay system to rapidly measure fractionated, radiation-induced fibrosis.

OHIO STATE UNIVERSITY (NO1-CM4-7622)

The principal objective of this project is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease during Phase I studies to help establish the most effective dosage schedule. Two new or established cancer chemotherapeutic agents are evaluated annually. The studies involve approximately thirty patients per drug. During this period the Contractor evaluated Acodazole and Nafidimide.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM3-7598)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor studied or is studying three drugs under separate task order contracts: Aphidicolin Glycinate (NSC 303812), Merbarone (NSC 336628), and Cyclodisone (NSC 348948). The contract was conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. This information is used to make decisions on further development of two drugs and in the case of Merbarone was used for IND filing and by by Phase I investigators in determining appropriate schedules for administration. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM5-7736)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. The Contractor is conducting and/or has completed Phase I studies with Nafidimide, Pibenzimol, and Flavone Acetic Acid.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CM6-7903)

Preclinical pharmacology studies of antitumor agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data as well as the efficiency of the Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to preclinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of antitumor agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because of the increasing emphasis on *in vitro* screens which is contemplated in the next several years. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed preclinically, the Phase I clinician is alerted to their potential human significance. This is a new Task Order Managed contract and replaces the previous Pharmacology Master Agreement.

ORI, INC. (NO1-CM3-7591)

This contract is intended to provide quick response programming support to enable the Information Technology Branch (ITB), DTP, to deal with the unanticipated problems or urgent requirements that are encountered in the operation of the ITB chemical and biological information system. Tasks have included, thus far, the transfer of the Hodes model to the Drug Information System (DIS) Pre-Registry subsystem, and Systems Analysis support for the DIS graphic subsystem (laser

printer) and the DIS data entry module. This contract, which was originally scheduled to expire September, 1986, was reawarded via the competitive process in May, 1986. The early award was necessary by an accelerated effort to meet Program needs.

ORI, INC. (NO1-CM5-7755)

This contract is intended to provide quick response programming support to enable the Information Technology Branch (ITB), DTP, to deal with the unanticipated problems or urgent requirements that are encountered in the operation of the ITB chemical and biological information system.

ORKAND CORPORATION (NO1-CM6-7716)

This contract supports the Clinical Oncology Program with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. A wide variety of systems have been developed and are maintained for the Clinical Branches of the Clinical Oncology Program.

PAN AMERICAN HEALTH ORGANIZATION (NO1-CM2-7391)

The Collaborative Cancer Treatment Research Program of U.S. and Latin American investigators is currently concentrating efforts towards Phase II studies in diseases such as gastric carcinoma, vulvar, penile and cervical squamous cell carcinomas very prevalent in Latin American Countries expected to generate important data in clinical oncology. Lesser numbers of Phase III studies are left at this point. NCI support for this contract will terminate in FY86, and currently there are no plans to continue the project.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM2-7529)

This Contractor contributes neutron data to a collaborative effort to improve treatment planning with heavy particles, to include protons, helium and heavy ions, and neutrons. In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, each contractor contributes patient data to other contractors that are members of the collaborative working group. Treatment plans developed by each contractor using the same patient data will be compared using evaluation methods developed by the group. A table of normal tissue tolerances for various organs has been developed by the working group.

PENNSYLVANIA, UNIVERSITY OF (NO1-CM4-7697)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

PHARM-ECO LABORATORIES, INC. (NO1-CM4-7587)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 50% of the effort of this contract is devoted to the preparation of large quantities of material, in the multikilogram range. The large-scale synthesis contracts are currently being recompeted under four separate RFP's.

PHYSICAL SCIENCES, INC. (N43-CM5-7834) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The purpose of this SBIR contract is to analyze the interaction of pulsed carbon dioxide lasers with biological tissue. Specifically, the Contractor has proposed to develop models that predict the thermal and/or pressure effects that then lead to tissue damage subsequent to laser irradiation and to identify experiments to test the validity of the models.

POLYSCIENCES,INC. (NO1-CM3-7557)

This service preparative contract provided for the large-scale extraction of various plants and isolation and purification of the active materials for pre-clinical development and clinical trials. Cost and yield data were obtained on these processes. In the case of those plant processes which will be used again, process development optimization studies were conducted. Materials processed include camptothecin, pancratistatin and taxol. This contract expired March, 1986 and has been replaced by a Master Agreement mechanism.

POLYSCIENCES, INC. (NO1-CM6-7707)

This Master Agreement for Master Agreement Orders in the large-scale isolation of antitumor agents from natural sources was established to permit the isolation and purification of active materials from organisms, such as plants and marine invertebrates, for use in preclinical development and clinical trials. This Master Agreement Order mechanism enables the prompt solicitation and award of projects to be conducted in this area from a pool of qualified contractors.

PROGRAM RESOURCES, INC. (NO1-CO2-3910)

This contract is located at the Frederick Cancer Research Facility in Frederick, Maryland. This contract is divided into the following eight segments:

Administrative Support

Provides for one administrative specialist, one senior clerk typist, and one information specialist in the Biological Testing Branch (BTB), Developmental Therapeutics Program (DTP).

Genetic Monitoring

Monitors all of BTB contract rat colonies for genetic purity. Also monitors the starts received from VRB-NIH, both pre and post rederivation.

In Vitro Cell Line Screening Project

This new project (began in September, 1984) is devoted to the development and operation of in vitro disease-oriented antitumor drug screening models based on the use of established human tumor cell lines. This activity is planned to represent the primary DTP drug screening effort and will interface with other components of the drug development program.

Microencapsulation Technology

This project will be terminated after a single 6-month period. An in vivo model for experimental human tumor chemotherapy evaluation will be perfected within this time period.

Models Development

This activity is closely related to the in vitro cell line screening project described above. Selected human tumor cell lines are being developed for use as models for in vivo drug evaluations.

Partial Support for Harlan/Sprague-Dawley

Provides Work Orders and Shared Services type functions for the Harlan/Sprague Dawley Animal Production contract. It serves as a method for paying PRI for services performed at the Animal Production Area.

Tumor Bank

This segment has as its major goal the maintenance of approximately 20,000 frozen tumor vials. This Contractor furnishes needed tumors to the various DTP laboratories, as well as to other research institutions, both domestic and foreign. The tumors are supplied both in vivo and in vitro.

Tumor Procurement and Preparation

Tumors will be procured from various surgical sites. Initial procurement efforts are being undertaken at Johns Hopkins University. The tumors will be adapted for the in vitro/in vivo DTP screening effort.

QUANTECH ASSOCIATES (N43-CM5-7810) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This contract is for the development of interactive statistical software to facilitate the use of state-of-the-art methodology in cancer clinical trials. Most mainframe statistical packages are of limited power and do not incorporate advanced interactive graphics or advanced statistical methodology. The S system developed at Bell Laboratories incorporates advanced interactive graphics and exploratory data analysis, but it does not include much of the methodology used in clinical oncology nor many recent methodologic developments. The objective of this contract is to develop a highly interactive system like S which includes the methodology utilized in clinical trials (e.g., logistic and proportional hazards regression) and which also facilitates the use of newer techniques such as cross-validation, bootstrapping, recursive partitioning and regression diagnostics.

RADIATION MONITORING DEVICES, INC. (N43-CM5-7807) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor proposes to investigate the use of a new, highly sensitive solid state x-ray sensor as the basis for an improved exposure controller. This sensor should make possible the construction of an automatic exposure controller for future mammography x-ray machines.

RADIATION MONITORING DEVICES, INC. (N43-CM5-7842) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This SBIR Contractor proposes to develop a working model of a computer controlled multi-leaf collimator designed to be mounted on existing medical accelerators currently used in radiotherapy. The company expects to produce a small working model of the device and develop conceptual designs for hardware and software controls and the optical reader to be interfaced to the collimator.

RAYLO CHEMICAL, LTD. (NO1-CM3-7635)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NO1-CM6-7698)

The objectives of this contract are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility, (b) increase compound stability; and (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles.

RESEARCH TRIANGLE INSTITUTE (NO1-CM3-7619)

Research Triangle Institute is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological and clinical phases of the Developmental Therapeutics Program. This Contractor determines the identity and purity of the compounds by appropriate methods. This Contractor also determines solubility, stability and other physicalchemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (paper, thin layer, gas liquid and high pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectroscopy and other methods as needed. Reports of the work performed by the contractor provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

RESEARCH TRIANGLE INSTITUTE (NO1-CM3-7636)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

RESEARCH TRIANGLE INSTITUTE (NO1-CM6-7703)

This service preparative contract provides for the synthesis of radioactive labeled chemicals and drugs for use in preclinical pharmacological and clinical studies. Many of the materials prepared are not available from commercial sources. All materials are analyzed for purity and identity by autography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by the National Cancer Institute staff.

SIMONSEN LABORATORIES (NO1-CM3-7624)

This contract is a Primary Genetic Center. Rederived associated flora pedigreed starts are supplied to this contract for propagation into isolator maintained foundation colonies. These foundation colonies supply breeders on a weekly basis to pedigreed expansion colonies which support the production colonies. Both the pedigreed expansion and production colonies are maintained in a barrier room environment. Offspring from the production colonies are used for hybrid production and the many NCI research activities. This contract effort is currently being recompeted.

SIMONSEN LABORATORIES (NO1-CM5-7729)

This Rodent Production Center contract produces two hybrids (B6C3F1, B6D2F1) under barrier conditions.

SISA, INC. (NO1-CM3-7637)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SITEK RESEARCH INSTITUTE (N43-CM5-7825) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

The objective of this contract was to develop human tumor cell lines with resistance to single or multiple standard chemotherapeutic agents for use in drug screening, drug mechanism of action studies and for studies of the problem of drug resistance and strategies to overcome it. This contract expired March, 1986.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CM3-7565)

The purpose of this contract is to provide methods for dose calculations for cancer therapy using radioactively labeled antibodies directed to tumor associated and/or tumor specific antigens. Dose calculations are to be developed for radiation doses to tumors and normal tissues resulting from the use of various isotopes (alpha, beta, or gamma emitters) tagged to antibodies for therapy purposes. Calculational methods are to be correlated with three-dimensional patient anatomy and the distribution and specific activity of radioactivity [as defined by scanners such as CT, single photon emission computed tomography (SPECT), positron emission tomography (PET) or gamma cameras] and should be compatable with conventional radiotherapy treatment planning systems insofar as possible.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CM5-7720)

This contract is for the production of murine monoclonal antibodies directed against human cytokines. This Contractor develops appropriate immunizing protocols to confirm the immunogenicity of the human cytokine in mice, and produces and isolates individual hybridoma clones secreting monoclonal antibody. Appropriate radioimmune assays are developed for screening individual hybridoma clones for antibody reactivity and ability of monoclonal antibody to specifically bind to and inhibit each cytokine. This Contractor provides anti-cytokine secreting hybridomas and semi-purified immunoglobulin derived from the various hybridomas to the National Cancer Institute for further distribution to qualified investigators. This Contractor also uses the antibodies to develop immunoassays for the cytokines in question. This Contractor has developed hybridomas secreting monoclonal antibodies against human IL-2, human gamma interferon and human alpha interferon and has undertaken development of monoclonal antibodies against human and murine tumor necrosis factor, human B-cell growth factor, interleukin-1, human beta interferon, and human and murine colony stimulating factors. This contract was awarded September, 1985.

SMALL BUSINESS ADMINISTRATION (BIOTECH RESEARCH LABORATORIES) (NO1-CM3-7609)

The purpose of this contract is to provide supportive services in virology, immunology, and tissue culture. At the present time, these functions include: (1) detailed karyotypic analysis, including Giemsa banding; (2) evaluation of tumorigenicity of various cultured cells by inoculation into nude mice; (3) preparing small quantities of selected cells and retroviruses; (4) testing various tissue cultured cell specimens for mycoplasma contamination; and (5) analyses of sera for HTLV-I or -III related antibodies in sera of patients and normal donors by ELISA and Western blotting techniques.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM2-5606)

This Contractor provides support services for conference management and associated general logistical activities for the Cancer Therapy Evaluation Program. Logistics support includes various technical and clerical tasks ranging from report design and preparation to routine typing. Conference support includes both pre- and post-conference management activities necessary to successfully conduct large as well as small meetings and provide the results thereof to the biomedical research community.

SOCIAL & SCIENTIFIC SYSTEMS, INC. (NO1-CM3-7576)

This Contractor provides technical assistance and support services in the area of investigational drug regulations and clinical research. Information is gathered and assembled for the preparation of Investigational New Drug Applications (IND's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the Food and Drug Administration, and an IND is awarded. This Contractor then maintains the files by providing in-depth tracking of drugs and amending IND information as necessary. This Contractor also provides drug distribution monitoring assistance and is involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information. This contract also provides support for the receipt, organization, and disbursement of all clinical protocols and related material involved in the CTEP protocol review process.

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM3-7639)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SOUTH FLORIDA, UNIVERSITY OF (NO1-CM5-7717)

The objectives of this contract are: 1) to develop and standardize methods of effectively screening human cytokines for direct and indirect anticancer effectiveness in vitro utilizing human cells as effector cells and several different human tumor cells as targets; 2) for human cytokines that are not species specific, to develop methods of testing the effectiveness of in vivo administration in animal models to: (a) modify the ability of cancer bearing host to react to its tumor and (b) effect restoration of depressed immune reactivity caused by chemotherapeutic or radiotherapeutic treatment procedures or by the tumor itself; and 3) to evaluate human cytokines supplied by the Biological Response Modifiers Program in the above mentioned screening procedures. This Contractor will perform core assays on all cytokines including NK/LAK cell cytotoxicities, mitogen stimulation, AMLR-T cell cytotoxicity, monocyte cytotoxicity, PMN inhibition of candida growth and effects on tumor cell growth. In addition, specialized assays will be performed depending on the properties of individual cytokines. This contract was awarded in FY 1985.

SOUTHERN ANIMAL FARMS (NO1-CM5-7727)

This Rodent Production Center contract produces CD2F1 hybrid mice and the CD8F1 hybrid. In addition, the pure strains BALB/c-CMc and DBA/8 are maintained within this contract. This contract terminated December, 1985.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CM3-7600)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor is currently working on Clomesone (NSC 338947) a drug currently under development by DTP. This contract is being conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. The data collected will be used to make decisions on further development of this drug. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

SOUTHERN RESEARCH INSTITUTE (NO1-CM3-7552)

This contract utilizes a variety of in vivo murine leukemia and solid tumor models to evaluate the antitumor activity of congeners or prodrugs of new lead compounds. Structure-activity studies are conducted under well controlled experimental conditions in order to guide future synthetic efforts and identify the most promising members of a class for further development. In addition, studies are conducted to evaluate the antitumor activity of purified natural products and to further explore the activity of selected congeners, as needed, against tumors with acquired resistance to clinical agents, against tumors implanted at various sites or by different routes of administration. This contract expired February, 1986.

SOUTHERN RESEARCH INSTITUTE (NO1-CM3-7638)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7580)

The objective of this contract is to conduct detailed preclinical studies on new antitumor agents identified by primary screening in order to optimize drug activity and evaluate the agent's therapeutic potential. As appropriate, studies are conducted in vitro or in vivo against transplantable murine tumors and human tumor xenografts. Tasks include evaluation of the effect of tumor site on drug activity, the effect of drug on advanced tumors, and the influence of drug schedule and route on activity. Additionally, studies are conducted to determine activity of formulated products, drug-resistance profiles and synergistic potential of drugs used in combination. This contract is being recompeted.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7581)

This contract provides assistance to the Biological Testing Branch, DTP, in protocol development and quality control of tumor lines and host animals. Tentative protocols are developed for new test systems or refinements suggested to enhance current systems. Animals and tumors used in the screening program are monitored for reproducibility and consistency of response. Cell kinetics are elucidated for in vivo systems utilized by the screening program.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7615)

The objective of this contract is to provide preliminary information on the cytotoxic and biochemical effects of new antitumor agents being considered for development to clinical trial. In vitro experiments are conducted (1) to establish whether agents with novel chemical structures have biochemical activities similar to those of clinically evaluated drugs; (2) to answer specific biochemical questions on new antitumor agents that are raised by the Decision Network Committee; and (3) to determine the drug concentration and time of exposure required for significant tumor cell kill. The latter information is used to help guide treatment schedule selection and dose escalations for Phase I clinical trials. This contract was phased out September, 1986.

SOUTHERN RESEARCH INSTITUTE (NO1-CM4-7646)

This contract provides for the testing of materials in the P388 pre-screen and the mouse host antitumor models in the DCT panel of in vivo test systems at a level of 18,000 L1210 equivalents per year. This contract provides for special studies as requested by the Project Officer such as the detailed evaluation of compounds and the characterization and evaluation of tumor models. All testing is carried out according to the protocols of the Biological Testing Branch, DTP. Conventional mouse tumor panel models now in use at this laboratory are the L1210 leukemia and the M5076 sarcoma mouse tumor test systems. Human tumors include the LOX amelanotic melanoma and the 3C2 colon tumor.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7724)

This contract was awarded to Southern Research Institute in February, 1986 following recompetition of contract number NO1-CM3-7552. The objective of this contract is to evaluate in vivo the antitumor activity of congeners of new lead compounds in an effort to identify the most promising of a structural class and to guide future synthetic efforts. It also provides for the evaluation of purified natural products. Special studies, such as the evaluation of experimental clinical formulations, the effects of route and schedule of drug administration, and the activity of drugs against tumor models with acquired resistance may also be carried out.

SOUTHERN RESEARCH INSTITUTE (NO1-CM6-7905)

Preclinical pharmacology studies of antitumor agents under development by DTP are conducted under this contract to collect data which will be used to improve both the interpretation of preclinical toxicology data as well as the efficiency of the Phase I trials of new agents. Task Orders are issued to contractors to perform defined pharmacologic projects. In general these studies are conducted in parallel with or prior to preclinical toxicology evaluations of the experimental agents. It is the expectation that from these studies will come assays of antitumor agents of sufficient sensitivity to be used in the clinic. Further studies will be designed to investigate the pharmacokinetics of the drug after bolus and infusion dosing in mice, rats and/or dogs. A major objective of this project is the collection of data which will ultimately lead to an understanding of species differences so that a significant reduction in the number of doses may result in the dose escalation schemes employed in Phase I clinical trials. The pharmacological information obtained through the Task Orders will play a potentially greater role in the drug development process in the future because

of the increasing emphasis on in vitro screens which is contemplated in the next several years. Basic pharmacokinetic data will contribute to the planning of treatment regimens and when significant species differences are observed preclinically, the Phase I clinician is alerted to their potential human significance. This is a new Task Order Managed contract and replaces the previous Pharmacology Master Agreement.

SOUTHWEST RESEARCH INSTITUTE (NO1-CM3-7640)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SRA TECHNOLOGIES, INC. (NO1-CM6-7750)

A contract has been awarded to a minority firm, SRA Technologies, Inc., of Alexandria, Virginia, to provide data management and statistical analysis services to NCI in support of the MRI Collaborative Working Group of five contractors who are comparing the diagnostic accuracy of magnetic resonance imaging vs. X-Ray CT for several parts of the body.

SRI INTERNATIONAL (NO1-CM3-7605)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor studied the compound L-Cysteine Derivative (NSC 303861). This contract was conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. The information from Phase I and Phase II is available for future use if interest in this drug is reinstated. Phase III was not conducted because decisions were made to stop development of this compound. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

SRI INTERNATIONAL (NO1-CM3-7618)

SRI International is one of the three contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological and clinical phases of the Developmental Therapeutics Program. This Contractor determines the identity and purity of the compounds by appropriate methods. This Contractor also determines solubility, stability and other physical-chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (paper, thin layer, gas liquid and high pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry and other methods as needed. Reports of the work performed by the contractor provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

SRI INTERNATIONAL (NO1-CM3-7641)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

SRI INTERNATIONAL (NO1-CM4-7611)

The objective of this contract between NCI, SRI International and Stanford University is the design, synthesis and biological evaluation of novel radiosensitizers. The primary focus of the work is the identification of leads other than electron affinic-2-nitroimidazoles. Other types of compounds that are being investigated include inhibitors of the repair of potential lethal damage, shoulder modifiers and glutathione depleters.

STANFORD UNIVERSITY (NO1-CM1-7480)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Center for Devices and Radiological Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

STARKS ASSOCIATES, INC. (NO1-CM3-7642)

This is one of the 10 master contractors for the synthesis of a variety of organic and/or inorganic compounds which have been identified by program for development. Unique compounds of interest emerging from the literature that cannot be obtained in sufficient quantities from the original investigators are synthesized by this mechanism. This mechanism is also used for the resynthesis of a limited number of panel compounds, and compounds of interest to intramural scientists. Potential radiosensitizers and radioprotectors are also synthesized in support of the Radiation Research Program.

STARKS ASSOCIATES, INC. (NO1-CM4-7588)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 50% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities and/or quality needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale. The large-scale synthesis contracts are currently being recompeted under four separate RFPs.

STARKS C. P. (NO1-CM4-7608)

This contract is in support of the Drug Synthesis and Chemistry Branch's fundamental responsibility to acquire selected novel synthetic compounds for evaluation as potential anticancer agents - the initial step in the National Cancer Institute's Linear Array for drug development. The major focus of this contract is the active solicitation, acquisition, documentation and management of the flow of approximately 10,000 compounds per year of diverse structural types. These compounds are selected by the Branch from a much larger pool of compounds provided through this contract in quantities adequate for the primary anticancer screen. This contract also acquires a significant proportion of the larger samples needed for secondary screening of the many new leads that are identified.

STATE UNIVERSITY OF NEW YORK (NO1-CM2-7570)

This project is one of the three contracts whose objectives are to design and synthesize the following: (1) congeners of lead compounds to enhance the activity or broaden the antitumor spectrum; (2) "pro-drugs" that are chemically altered transport forms of compounds to modify both biological and pharmaceutical properties, such as (a) improve bio-availability by increasing aqueous solubility, (b) increase compound stability; and (3) compounds related to products of natural origin and other heterocycles with improved antitumor activity and decreased toxicity. These modifications include partial structures, structural analogs and novel heterocycles.

T AND B BIOCLONE (N43-CM5-7779) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Monoclonal antibodies specific for a human lung tumor-associated glycoprotein antigen (gp-160) will be used to develop and test a cytotoxic drug delivery system for therapy of cancer patients and to develop simple diagnostic/prognostic immunoassays. The gp-160 membrane-associated glycoprotein that is found in significant levels on several major types of human lung tumors including squamous cell carcinomas, adenocarcinomas and large cell tumors. The specificity of a monoclonal antibody (5E8) for the tumor-associated antigen has been established. The proposed therapeutic exploitation involves covalent coupling of these monoclonal antibodies to unilamellar liposomes that are filled with a cytotoxic drug. Recent advances in the preparation of liposomes have been made and the successful therapeutic exploitation of such immunospecific liposomes has been established in a mouse tumor model. Phase one of this proposal will include: (1) preparation of gp-160 specific drug-containing liposomes; (2) a test of the ability of immunospecific liposomes to kill gp-160 positive human lung tumors selectively in vitro; (3) generation and purification of 25-50 grams of gp-160 specific antibodies; and (4) establishment of simple and sensitive immunoassays for detecting circulating gp-160 or anti-gp-160 in lung tumor patients.

TACAN CORPORATION (N43-CM5-7859) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This SBIR Contractor proposes to develop a system that produces images from laser-induced fluorescence in tissue injected with hematoporphyrin derivative (HPD). The compact, multicolor imaging system will also be able to achieve strong contrast enhancement of the tumor by monitoring fluorescence intensities in selected wavelength bands. Using fiber optics to transmit images from deep-seated tumors using endoscopic techniques, the Contractor expects to provide a unique capability in tumor localization for patients requiring surgical excision.

TACONIC FARMS (NO1-CM5-7730)

This Rodent Production Center contract produces athymic nude mice under maximum barrier conditions.

TECHNICAL RESOURCES, INC. (NO1-CM5-7658)

This project provides technical support services to the Office of the Director, DCT, as well as to the program areas of DCT in the performance of the planning and analytical tasks and general logisticial support in the development of related or otherwise required documentation and conference support activities of the Division. This contract was awarded in April, 1985 for a five-year period.

TECHNICAL RESOURCES, INC. (NO1-CM6-7907)

This contract was awarded to this SBA organization in May, 1986 as a result of the recompetition of contract NO1-CM3-7558 with Biotech Research Laboratories, Inc. The new award reflects a broadened workscope to provide support assistance in a variety of operational and management tasks relating to the Developmental Therapeutics contract, grant and cooperative drug discovery group programs.

TECHNISCAN, INC. (N43-CM5-7805) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

A new fast computer algorithm has been developed and demonstrated which permits ultrasound imaging and tissue characterization quantitatively with high spatial resolution (near one-half wave length, i.e., 1/2 mm) and free of the image distortions caused by refraction (bending) and diffraction (beam spreading) of the ultrasonic beam as it passes through tissues. Exact solutions of the Helmholtz wave equation have been achieved to solve the inverse scattering problem. Significant improvements over non-quantitative B-scan technology using phantom models have been shown. Application of this improved technology will lead toward high quality, high resolution ultrasound diagnostic images in future systems. Work has begun using a Cray XMP/48 supercomputer to provide high-speed computations in undistorted image reconstruction.

TEGERIS LABORATORIES, INC. (N43-CM5-7822) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

During a Phase I SBIR contract, this organization investigated prodrugs and liposomes as a means to enhance the deliverability and stability of selected antitumor agents. More lipid soluble prodrugs were prepared and then incorporated into the lipid phase of emulsions or liposomes as a means of reducing the decomposition rate. This contract expired March, 1986.

TEXAS A&M RESEARCH FOUNDATION (NO1-CM3-7536)

This contract montitors the genetic purity of the strains produced at the Genetic Centers and Rodent Production Centers. The testing is done by checking biochemical markers, and animals are sent for monitoring on a weekly basis scheduled by the Project Officer.

TEXAS, UNIVERSITY OF (NO1-CM5-7775)

This Contractor is part of a collaborative working group to conduct Phase III randomized clinical trials and Phase II studies of fast neutrons versus photons for tumors which are not presently controlled by conventional radiotherapy in the major anatomical sites. The working group meets twice each year to design protocols, review the quality control of each contractor through subcommittees, and collaborate with other neutron contractors on state-of-the-art neutron treatment techniques. Phase III protocols are being opened for randomized studies to compare neutrons with photons as they are approved by the working group and the NCI. Currently approved are three protocols for a) patients with squamous cell or lymphoepithelioma carcinoma of the upper aero-digestive tract, b) adenocarcinoma of the prostate gland, and c) cancer of the uterine cervix.

TEXAS, UNIVERSITY OF, HEALTH SCIENCE CENTER (NO1-CM5-7737)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with Tiazofurin, Mitoxantrone, Fludarabine, Didemnin-B, Nafidimide, and Flavone Acetic Acid.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM1-7524)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Center for Devices and Radiological Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM2-7531)

This Contractor contributes neutron data to a collaborative effort to improve treatment planning with heavy particles, to include protons, helium and heavy ions, and neutrons. In addition to developing optimal treatment plans for the treatment of tumors in various anatomic sites with the particle beam at its own institution, each contractor contributes patient data to other contractors that are members of the collaborative working group. Treatment plans developed by each contractor using the same patient data will be compared using evaluation methods developed by the group. A table of normal tissue tolerances for various organs has been developed by the working group.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CM5-7739)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment, Phase II/III studies in patients with disseminated solid tumors and leukemia, or Phase I clinical studies with drug combinations or bone marrow transplantation as

mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with BIDA, Trimetrexate, Ifosfamide with Mesna and Methotrexate, VP-16 and Methyl-GAG and Phase II/III studies of Ifosfamide with the Chemoprotector Mesna in unresectable non-small cell and extensive small cell lung cancer.

THERADEX SYSTEMS, INC. (NO1-CM3-7553)

The objective of this contract is to provide a Clinical Trials Monitoring Service for the Phase I/II CTEP and BRMP investigators and all other investigators using NCI-sponsored investigational drugs. This service has four components: (1) a central data management resource for investigators conducting Phase I clinical trials; (2) a site visit monitoring resource for DCT to assure that Phase I/II contractors are in compliance with federal regulations; (3) co-site visiting cooperative groups as observers of peer audits; and (4) site visit monitoring of all other individual investigators conducting investigational drug trials.

THERAPEUTIC DELIVERY SYSTEMS (N43-CM5-7797) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

In this proposal we intend to specifically investigate the potential for liposome encapsulation of lymphotoxin and its delivery to specific liver cells where lymphotoxin can augment a natural response against tumor. Understanding how the host's natural defense system can be modulated by chemotherapeutic drugs and BRM in a number of pathologic states is critical in determining their clinical usefulness. Since the liver is a major target of invasion by metastatic tumors, we have selected it as a "model" organ to accomplish our research aims. We have developed, and will employ in this project, techniques to study the liver's natural and anti-tumor defense system in vitro/ex vitro as well as methods to target immunoenhancing agents to various regions of the murine liver using liposomes of varied size and composition.

THERAPEUTIC TECHNOLOGIES, INC. (N43-CM5-7850) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Under this Phase I SBIR contract, new prodrug derivatives were evaluated as a means to improve deliverability of selected antitumor agents. The solubility and stability behavior was studied using standard pharmaceutical protocols. This contract terminatd May, 1986.

THERMAL TECHNOLOGIES, INC. (N43-CM5-7855) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor proposes to combine two separate thermal probe methods to produce a simultaneous, integrated methodology for the local transient quantification of tissue thermal conductivity, diffusivity, and perfusion. Each technique employs a thermistor probe, embedded in tissue, to deduce thermal properties. The novel point in this proposed study is the observation that each technique is compatible with and complementary to the other. One experimental procedure then contains in effect two experiments, each of which may be optimized so as to simultaneously quantify tissue conduction and blood flow. The necessary performance criteria, experimental protocol, and data analysis procedure for an optimal technique for measuring tissue temperature and blood flow will be established.

TRANSAMERICAN IMMUNOLOGY (N43-CM5-7853) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Presence or emergence of tumor cells resistant to one or more chemotherapeutic agents is a common problem encountered in cancer patients. There is an urgent need for extensive study of the mechanism and development of methods to prevent drug resistance. We will be conducting a systematic study of clones generated from human tumor tissue in attempts to determine surface marker(s) characteristic of one or the multiple drug resistant phenotype. Choice of the human tumors is especially important since studies in hamster and murine systems do not seem to correlate with human tumors. We will also be making monoclonal antibody(s) in attempts to eliminate the resistant population by antibody alone, or antibody-conjugated chemotherapeutic agents which can also be used in the identification of resistant populations.

TRIANGLE RESEARCH AND DEVELOPMENT CORPORATION (N43-CM5-7856) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This SBIR Contractor proposes to develop and test a model of a computer-controlled multi-leafed collimator for radiotherapy accelerators. The company has proposed to design and fabricate a multi-leaf assembly of five opposing pairs of leaves and to investigate optimal requirements for positioning accuracy, response times, patient input, retrofit considerations to existing machines, reliability, operation and cost.

TRIPOS ASSOCIATES (N43-CM5-7846) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This contract is for the development of interactive statistical software to facilitate the use of state-of-the-art methodology in cancer clinical trials. Most mainframe statistical packages are of limited power and do not incorporate advanced interactive graphics or advanced statistical methodology. The S system developed at Bell Laboratories incorporates advanced interactive graphics and exploratory data analysis, but it does not include much of the methodology used in clinical oncology nor many recent methodologic developments. The objective of this contract is to develop a highly interactive system like S which includes the methodology utilized in clinical trials (e.g., logistic and proportional hazards regression) and which also facilitates the use of newer techniques such as cross-validation, bootstrapping, recursive partitioning and regression diagnostics.

ULTRATHERMICS, INC. (N43-CM5-7839) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Contractor is developing a non-invasive hyperthermia system that features a novel treatment applicator with the ability to dynamically vary both the spatial and temporal disposition of ultrasonic energy in response to measured temperature data. The purpose of this Phase I contract is to demonstrate the feasibility of the proposed system by modeling its heating performance when applied to a number of clinically significant situations. It is anticipated that the results of this study will reveal those operational parameters which have the greatest impact on heating, identify effective scanning/heating protocols, clarify the physiological and engineering limitations of the system, and culminate in a preliminary product specification document.

UTAH, UNIVERSITY OF (NO1-CM1-7523)

This contract is for the Phase I evaluation of equipment for the hyperthermic treatment of cancer in conjunction with radiotherapy, chemotherapy, surgery, or immunotherapy. Five contractors are collaborating in a working group in conjunction with the Center for Devices and Radiological Health to develop guidelines for the use of heat generating and thermometry equipment to facilitate the development of Phase II and III clinical studies for the adjuvant treatment of deeply seated tumors with heat.

VERMONT, UNIVERSITY OF (NO1-CM2-7547)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with new combinations of regimens mutually agreed upon. This Contractor conducted Phase I studies with Spiromustine and with Didemnin-B. This contract expired January, 1986.

VERMONT REGIONAL CANCER CENTER (NO1-CM3-7606)

Under a Master Agreement for pharmacology task orders to conduct preclinical studies of the pharmacology of new or established antitumor agents, this Contractor is studying Chloroquinoxaline Sulphonamide (NSC 339004). The contract is being conducted in three phases: Phase I, analytical methods development; Phase II, bolus dose pharmacokinetics; Phase III, infusion dose pharmacokinetics. The information will be used for making decisions on further drug development and if appropriate for IND filing and by Phase I investigators in determining appropriate schedules for administration. This Master Agreement expired January, 1986 and is being recompeted as a Task Order Managed contract.

VESTAR RESEARCH, INC. (N43-CM5-7794) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Many immmunostimulatory agents are available which are potential macrophage activating agents. However, their in vivo ability to induce antitumor activity in macrophage is severely limited due to the rapid clearance of these agents. This project proposes to use phospholipid vesicles (liposomes) as a delivery system for macrophage activating factors. The liposome delivery system will enhance the efficacy of these agents by extending their time in circulation and by increasing the amount of agent delivered to macrophage. Phase I will demonstrate a liposome formulation which satisfies the objectives of prolonged biological lifetimes for the activating agents and increased delivery to blood monocytes and pulmonary and hepatic macrophage.

VESTAR RESEARCH, INC. (N43-CM5-7823) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

This Phase I SBIR contract evaluated the feasibility of liposomal encapsulation in the formulation of selected antitumor agents. This Contractor also evaluated the effect of liposomes on the chemical stability of these compounds and the compatibility of these drug-loaded vesicles with blood and serum in vitro. This contract expired March, 1986.

VSE CORPORATION (NO1-CM5-7654)

This contract is in the second year of a five year period. Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes: (1) reducing and disseminating data resulting from the screening program of the Drug Evaluation Branch to staff and suppliers of the compounds being tested; (2) writing new computer programs to process data from new methods of screening; (3) maintaining and modifying existing computer programs so that they are able to run at the Division of Computer Research and Technology; (4) improving data collection methods; (5) documenting all computer programs and Contractor's procedures for data handling and running computer programs; compounds relating to collection and dissemination of data; (7) performing statistical analyses of test systems and test system parameters; (8) extensively collaborating with the Drug Information System Contractor; and (9) participating in scientific meetings.

WARNER-LAMBERT COMPANY (NO1-CM3-7285)

This no-cost contract provides for developing and marketing AZQ as an antitumor agent. Its purpose is to facilitate development of the drug to the NDA stage. Warner-Lambert has conducted both pre-clinical and clinical studies with AZQ. All the additional toxicology studies required for FDA approval and foreign registration have been completed or will be initiated soon. Warner-Lambert is presently collecting data from two studies which compare AZQ with BCNU in patients with primary brain tumors.

WARNER-LAMBERT COMPANY (NO1-CM4-7589)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts and/or quality required. About 30% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities. The large-scale synthesis contracts are currently being recompeted under four separate RFP's.

WARNER-LAMBERT COMPANY (NO1-CM3-7614)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermented cultures from various microorganisms isolated under a bevy of environmental and stress conditions from unique substrates from various parts of the world; (2) tissue culture assay which has been semi-automated to increase the screening of new cultures for cytotoxicity, accelerate the fractionation of new leads and facilitate fermentation optimization and scale-up production of new agents; (3) dereplication of active components to verify their novelty using new techniques, including reverse phase silica gel chromatographic methods; (4) isolation and purification of new compounds with the use of high pressure liquid chromatography; and (5) large-scale production of lead compounds for advanced development. Twenty-two novel compounds have been isolated and submitted to NCI for antitumor evaluation. An IND application is being prepared for Fostriecin and large quantities of this compound have been prepared for Phase I trials. Formulation and stability studies on Elactocin have been completed and large quantities of the compound have been prepared for further development. A back-up compound, Hydroxyelactocin was also prepared.

Two other compounds have been isolated which are novel and extremely potent with broad spectrum antitumor activity. This contract was completed January, 1986.

WASHINGTON UNIVERSITY (NO1-CM4-7696)

The objective of this collaborative effort is to develop criteria, guidelines, and methodology for the performance and evaluation of state-of-the-art high energy photon external beam treatment planning. This will be accomplished by extensive treatment planning for actual patients and by using state-of-the-art beam delivery, computerized treatment planning, and imaging systems. The results of this work will be described in a final report which will define state-of-the-art treatment planning and provide criteria, guidelines, and methodology for its application and evaluation.

WASHINGTON, UNIVERSITY OF (NO1-CM9-7282)

This contract provides a cyclotron-based neutron therapy system, a clinical facility in which to house the equipment and personnel to support a clinical neutron therapy research program at the University of Washington. The facility construction was completed in March, 1984. The cyclotron was accepted in September, 1984 and patient treatment began in November, 1984. Phase III protocols are being opened for randomized studies to compare neutrons with photons as they are approved by the working group and the NCI. Currently approved are three protocols for a) patients with squamous cell or lymphoepithelioma carcinoma of the upper aero-digestive tract, b) adenocarcinoma of the prostate gland, and c) cancer of the uterine cervix.

WAYNE STATE UNIVERSITY (NO1-CMO-7404)

This Contractor conducts Phase II/III studies in patients with advanced solid tumors. A minimum of 200 patients a year were studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the NCI and the Principal Investigator. Treatment and follow-up of patients ended in February, 1986.

WEST INDIES, UNIVERSITY OF (NO1-CP3-1006)

An interdivisional transfer of funds to DCE, Environmental Epidemiology Branch, to support clinical and treatment investigations in patients with Adult T Cell leukemia/lymphoma (ATL) using NCI approved clinical studies. The objective of this Task is to evaluate the efficacy of an aggressive combination chemotherapeutic program supplemented by immunotherapy in the management of patients with ATL. This will be achieved by using P-COLADA (prednisone, cyclophosphamide, vincristine, high-dose methotrexate with leucovorin rescue, adriamycin and deoxycoformicin) to induce complete remission, and prolonging disease-free survival by administering anti-Tac to those patients who have achieved complete remission. Anti-Tac is an antibody directed against the receptor for T-cell growth factor. Previously treated patients who have relapsed or are refractory to standard chemotherapy will be eligible for a Phase II deoxycoformycin study.

WISCONSIN, UNIVERSITY OF (NO1-CMO-7434)

This Contractor is conducting three Phase I/II clinical trials of recombinant gamma interferon in the treatment of patients with disseminated malignancies. The final study of this contract is comparing the biologic response and clinical toxicity at doses of 3, 10, 30, and 300 MU/m^2 as rapid intravenous infusions. Extensive clinical and immune monitoring is being performed to determine an optimal biological response modifying dose. This contract expired June, 1986.

WISCONSIN, UNIVERSITY OF (NO1-CM4-7669)

Under a Master Agreement, a Phase Ia trial is investigating a single, rapid infusion versus a 24-hour continuous infusion of recombinant interleukin-2 in patients with advanced malignancies. While immunological responses are being monitored, the major aim of this study is to determine the maximal tolerated dose. Clinical toxicity has included hypotension, fever, chills, and fluid accumulation, which was significant at doses of 10^7 units/m^2/day. Immunologic and pharmacokinetic data is being analyzed.

In addition, another trial is being conducted of recombinant interleukin-2. Based on results from the previous Phase Ia trial, dose and schedule manipulations will be further explored to determine an optimal biologic response modifying dose.

In addition, another trial is examining the biologic efficacy and clinical toxicity of recombinant interleukin-2 and lymphokine activated killer (LAK) cells in patients with advanced malignant disease. Patients will be closely monitored for clinical toxicity and immunological response. Comparisons will be made between autologous and allogeneic LAK cells, as well as the effects of pretreatment with cyclophosphamide.

In addition, another trial is being conducted of the combination of recombinant interferons gamma and beta-serine. Clinical toxicity and biological response will be monitored, as well as antibodies to the interferons and interferon levels. This trial began in early Spring.

WISCONSIN, UNIVERSITY OF (NO1-CM5-7735)

This contract is designed to conduct Phase I and clinical pharmacokinetic studies with new anticancer agents sponsored by the Division of Cancer Treatment or to conduct Phase I clinical studies with drug combinations or bone marrow transplantation as mutually agreed upon. This Contractor is conducting and/or has completed Phase I studies with Acodazole HCL, Acivicin and Dipyridamole, Carboplatin and Cisplatin, Taxol, and Recombinant Human Tumor Necrosis Factor (RTNF).

XOMA CORPORATION (N43-CM5-7843) (SMALL BUSINESS INNOVATIVE RESEARCH Program)

Fifteen iron and gadolinium pharmaceutical complexes have been prepared and evaluated for safety and efficacy as potential contrast enhancement agents for magnetic resonance imaging (MRI). On the basis of in vitro tests, acute toxicity studies, and imaging experiments in mice, two chelates (Fe-EHPG and Fe-HBED) appear to be safe, undergoing hepatobiliary excretion and effective as indicators of hepatic function and as MRI contrast agents. In additional Phase I studies seeking to develop "targeted" agents for receptor-specific interactions, an

antimelanoma monoclonal antibody has been covalently linked to a metal ion chelating group, and the magnetic relaxivities of iron and gadolinium compounds have been determined.

YALE UNIVERSITY (NO1-CM4-7681)

This Contractor will conduct a Phase Ia/Ib trial of recombinant interferons alpha and gamma. Special consideration to study design is being given in order to fully evaluate the interaction of the two interferons. Several doses of each interferon will be evaluated for clinical toxicity and immunologic effect. This trial began in early Spring. This contract will expire December, 1986.

YALE UNIVERSITY (NO1-CM5-7777)

The objective of this collaborative effort is to develop recommendations and guidelines for a program in Interstitial Radiotherapy. The recommendations shall include calibration and dosimetry of clinical radioisotope sources, software packages for calculation, a quality assurance program, and the clinical use of interstitial radiotherapy. The latter shall include recommendations for tumor sites that would benefit from interstitial radiotherapy implant techniques, dose rate and dose distribution, safety precautions and afterloading procedures where appropriate.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 - September 30, 1986

I. Introduction

The Developmental Therapeutics Program (DTP) has primary operational responsibility for all aspects of the preclinical development of antitumor drugs for the Division of Cancer Treatment (DCT). The extramural component of the DTP is located in the Landow Building in Bethesda, Maryland, where directed drug development activities are contract-supported and research in biochemistry and pharmacology is administered through the grants mechanism. The DTP intramural laboratory operation conducts anticancer drug and other research related to preclinical cancer treatment in Building 37 on the NIH campus in Bethesda.

The extramural program, which is devoted to the acquisition, antitumor evaluation, formulation, pharmacology and toxicology studies and large-scale drug production of new candidate anticancer drugs, is managed by seven Branches: Drug Synthesis and Chemistry, Natural Products, Biological Testing, Information Technology, Pharmacology, Toxicology, and Pharmaceutical Resources. An eighth Unit, the Office of Extramural Resource Analysis and Development, is responsible for the management of contracts, grants and cooperative agreements for the support of chemistry, biochemistry and pharmacology related to cancer.

The intramural program is conducted in six Laboratories: Biological Chemistry, Experimental Therapeutics and Metabolism, Medicinal Chemistry, Biochemical Pharmacology, Molecular Pharmacology, and Tumor Cell Biology. Intramural research supports both new drug studies and basic investigations in cancer-related biochemical processes and molecular biology.

The Office of the Associate Director is responsible for the leadership and management of the DTP and the accomplishment of the goals and objectives of the DCT preclinical program. The progress of potential clinical candidates through the Decision Network process is summarized in Table 1.

The major reorientation of the drug development program which was reported last year has seen substantial advances this year. At the beginning of the year a two-day conference was held to review the entire plan for the disease-oriented drug discovery effort, in which candidate drugs are to be screened *in vitro* against numerous human tumor cell lines. Experts from Europe and the U.S. attended, some of the latter being past or present members of the DCT Board of Scientific Counselors. The experimental design was discussed in great detail and the framework of an optimal experimental procedure was agreed upon. The meeting was very useful in that it helped to chart the course of the *in vitro* assay work for some months into the future. Acquisition and development of human tumor cell lines has con-

tinued since then and 16 such lines are now in routine use in the in vitro assay. A further 30 lines are still in development. A computer system for the acquisition and processing of data from the in vitro assay has been installed and is now operational. Development of in vivo assay procedures based upon the cell lines used in vitro is well advanced. It is expected that during the forthcoming year, a pilot study of the in vitro screening system will be completed and that an assessment of this approach will become possible. Meanwhile, acquisition of synthetic chemicals for testing has levelled off and the acquisition of natural products has been re-emphasized. A Pharmacology Branch has been established in the extramural program and undertakes the measurement of the pharmacodynamics of compounds which appear likely to enter clinical testing.

II. Accomplishments

A. Extramural Program

1. Acquisition of new materials as potential anticancer drugs

a. Drug Synthesis & Chemistry Branch (DS&CB)

The fundamental responsibility of the DS&CB is the acquisition and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents in the DTP. As the emphasis of the DTP has begun to shift from a compound-oriented approach to a disease-oriented strategy, the challenge for DS&CB has been to throttle back the flow of new compounds into the system, while maintaining the large number of cooperative suppliers. Accordingly, the flow of compounds into the P388 in vivo screen has been reduced to 20% of its former level and at the same time, several smaller groups of compounds have been identified for examination in the in vitro assay.

Meanwhile, the Branch has pursued those agents which have shown some noteworthy activity in the in vivo screens. Specifically, flavone acetic acid (NSC 347512) has reached DN-4 and tetraplatin (NSC 363812) is now at DN-3. A patent on tetraplatin is to be issued to the government, which in turn will issue licenses for its commercial development. Two synthetic compounds passed DN-2A this year and four passed DN-2B.

The DS&CB also maintains a capability in the synthetic area and in this connection has synthesized several radiolabelled compounds, such as flavone acetic acid, ipomeanol (NSC 349438), merbarone (NSC 336628), and dideoxycytidine (NSC 606170), for use in preclinical and clinical studies. In addition, ten cyclitol derivatives have been prepared for evaluation in the Laboratory of Biological Chemistry as inhibitors of phospholipid metabolism and, in a notable achievement, DS&CB designed and synthesized a soluble tetrazolium derivative which has greatly facilitated the in vitro assay.

The Branch continues to operate NCI's chemical repository and during the year has shipped more than 16,000 samples to various investigators around the world. Automation of the repository and shipping operations is now complete and has facilitated both operation and record-keeping of this function.

b. Natural Products Branch (NPB)

The central goals of the Natural Products Branch are (a) to acquire a wide variety of unique chemical compounds of natural origin through donations and contracts, (b) to conduct a world-wide program of literature surveillance to identify materials of interest for acquisition and testing, (c) to develop new derivatives and analogs of compounds of interest, and (d) to produce additional quantities of compounds under study to ensure a sufficient supply of material for detailed biological evaluation and subsequent developmental studies including pharmaceutics and toxicology.

Natural products acquisition efforts have been expanded this year and fall into four main areas. The discovery and development of antitumor agents from microbial sources is being actively pursued under three separate contracts. Isolation of various genera of fungi and cultivation of blue-green algae are activities supported by new contracts. The collection of plant and animal products has been augmented by means of contracts for the collection of shallow water and deep water marine specimens. Finally, the collection of plant materials from Asia, Africa and South America has been resumed.

Surveillance of the literature for natural products of interest continues. During this year, 476 new natural products were identified by this method and were subsequently acquired by the NCI. Two natural products, deoxyspergualin (NSC 356894) and didemnin-B (NSC 325319) entered clinical trials this year. Didemnin-B is the first marine animal product to be so employed.

A number of derivatives of indicine-N-oxide (NSC 132319) have been prepared in the hope that they will prove to be less hepatotoxic than the parent and similarly, several analogs of camptothecin (NSC 100880) have been produced in an effort to enhance the natural product's antitumor activity.

2. Biological Evaluation

a. Biological Testing Branch (BTB)

As the thrust of the DTP changes to a disease-oriented approach to drug discovery, the responsibilities of the Biological Testing Branch have been shifting. This Branch is taking the lead role in the development of the primary *in vitro* screens and the related secondary *in vivo* models. At the same time, BTB manages the *in vivo* testing that remains to be completed from earlier work based upon the P388 pre-screen.

Over 85 human tumor cell lines have been examined for inclusion in an in vitro panel. Approximately 50 of these meet the working criteria for use in the in vitro assay and to date, 16 human tumor cell lines have been adopted for routine use in this assay. These include 10 non-small cell lung cancer lines and six miscellaneous human tumor lines. All cell lines that are to be used in the in vitro assay are characterized in depth. Protocols have been established for a growth inhibition assay. Complete automation of this assay has been achieved in collaboration with the ITB and a new soluble staining reagent, which facilitates the assay greatly, has been developed jointly with the DS&CB.

Pilot scale screening was undertaken with these 16 cell lines and a group of 108 compounds randomly selected from the DTP repository. All 108 compounds were screened against the complete cell line panel and as expected, most of the compounds showed no activity, but some suggestions of selective cytotoxicity were seen. A variety of technical problems were encountered and resolved in the course of this experiment whose main outcome therefore was a working bioassay which showed signs of being an effective screen.

Plans are well advanced for secondary testing in vivo of compounds which show selective cytotoxicity in the in vitro panel. To this end, in vivo models involving the same cell lines are being developed. The methods under study include implantation of the tumor cells in the sub-renal capsule or in the right mainstem bronchus of athymic mice. In addition, microencapsulation of the cells is also undergoing evaluation. All three of these approaches promise to provide a useful seccondary in vivo assay method.

Methods for evaluating the ability of drugs to combat pulmonary metastases have been studied and a useful model of metastatic human cancer has been derived from the human LOX melanoma. It is hoped that models of this sort will form the basis of a practical bioassay method.

As the disease-oriented methods have been developed, conventional in vivo screening has continued, but at a reduced level. In the year ending March 31, 7,671 new synthetics and 245 new natural products were tested against murine P388 leukemia. Of these compounds, 353 met the criteria for confirmed activity and were assigned to the tumor panel. The scale of the P388 pre-screen has now been reduced by 80%; currently, compounds are entering the pre-screen at the rate of 2,000 per year. The changing needs of the program in terms of mice has led to appropriate adjustments in the animal supply program. New strains have been supplied and a substantial increase has been achieved in the numbers of athymic mice that are produced.

The increasing use of the NCI tumor repository by investigators around the world was creating an unforeseen workload in the repository, which is at the Frederick Cancer Research Facility. The work has now been delegated to contractors whose cost is largely defrayed by means of a payback system.

b. Pharmacology Branch (PB)

This new DTP Branch has as its central responsibility, the pharmacodynamic and therapeutic evaluation of compounds that, on the basis of biological evaluation, are felt worthy of further development towards clinical use. Pharmacology studies carried out in this context include the development of analytical methodology, studies of *in vitro* stability and plasma binding and detailed pharmacokinetics following either single bolus dosing or continuous administration. Such studies have been completed for 10 compounds with a further 5 still in progress.

Three drugs that were the subject of INDA filings this year, flavone acetic acid (NSC 347512), merbarone (NSC 336628), and deoxyspergualin (NSC 356894) received special attention. In conjunction with the Toxicology Branch, the Pharmaceutical Resources Branch, and staff from CTEP and from FDA, the PB has completed a full study for each of these drugs. This was successful in relating pharmacokinetics, toxicology and therapeutic effects for the drugs in question, and it is expected that it will be used in future as a general approach for the study of clinical candidates.

c. Information Technology Branch (ITB)

The responsibility of the Information Technology Branch is to provide support for data acquisition and data processing for the entire DTP.

In parallel with the other extramural Branches of the DTP, the Information Technology Branch has adapted in order to support the new disease-oriented approach to drug discovery and development. Implementation of the Drug Information System (DIS) by ITB is complete. The DIS is a large search system containing all the data from acquisition to biological testing, associated with the NCI screening program. Searching in the DIS is interactive and is carried out by end-users with no ITB intervention. Approximately 10,000 DIS transactions are completed each month by about 100 users. For the first time, scientists outside the government are able to use the system and staff from pharmaceutical companies and universities in the U.S. and Europe are registered users of the DIS.

Much of the DIS is concerned with acquisition and storage of chemicals and these functions, which are highly automated, are proving to be of value as the *in vitro* screening experiment is developed. There existed no capability however for acquisition

of data from the in vitro screening laboratories and consequently, the ITB has developed a data acquisition system for this purpose. This new system employs a network of microcomputers, operating in conjunction with one another and under the ultimate control of the NIH mainframe computers. All testing is initialized by the DIS but is carried out under the control of microcomputers. Data from the experiments are passed back to the mainframe automatically where a searchable database of in vitro test data within the DIS will receive them. The new system is now essentially complete; numerous minor tasks await completion, but data acquisition is proceeding and the mainframe database is currently being installed.

The dynamics of the full in vitro testing system are still crystallizing, but it is already clear that a number of manpower-intensive and rate-determining steps are present. To deal with these, ITB has begun to exploit the possibilities of robotics. A single arm robot has been acquired and now can conduct sample weighings at the rate of 500 samples per 24 hours. This procedure is completely automatic; the robot selects and opens sample tubes, weighs the sample into a second labelled tube, closes both tubes and compiles a file of sample identification number versus sample weight. Work is now in progress to develop procedures whereby the robot can measure the solubility of compounds in specific solvents and load the plates used in the in vitro assay.

The use of computer graphics in connection with screening data in general continues. The laser printer installed in the Landow Building now produces graphics upon command. This high-quality output includes chemical structures, letterheads, foreign languages (e.g. Japanese), and signatures as well as graphs of all types.

As the DTP effort in natural products acquisition is stepped up, the ITB is addressing new tasks in support of the collection and extraction work. A series of data management systems has been developed and involves some special challenges as much of the NPB-sponsored sample collection effort is carried out in remote areas of the world, beyond the reach of telecommunications networks.

The pharmacodynamic basis of current toxicology and pharmacology studies requires computer programs for the acquisition and analysis of pharmacokinetic data and work to develop such programs is in progress.

3. Drug Development

a. Toxicology Branch (TB)

The primary responsibility of the Toxicology Branch is the conduct of investigations of the hazards presented by antitumor agents to healthy organs in intact experimental animals. This

goal is pursued by (a) elucidation of the potential adverse effects of new anticancer agents and (b) acquisition and use of pharmacokinetic information for each drug. These two types of data permit more reliable extrapolation of toxic effects across species by means of the relationships between plasma drug levels and observed toxic effects. A more precise evaluation of a drug's schedule dependency is also derived from such studies.

An important function of this Branch is to compile INDAs for submission to FDA. This is done using procedures which have been agreed upon by both agencies and are set forth in a "Master File". A proposal for augmenting the Master File so that it will in the future include pharmacokinetic data, has been prepared by TB and submitted to FDA. Four drugs whose toxicology studies followed the existing protocols were presented to the Decision Network Committee and studies have been initiated on a further 4 new antineoplastic agents. With the latter 4 agents, full pharmacokinetic data are being acquired, along with the toxicology data.

The DTP role in the development of anti-AIDS drugs is steadily increasing and the Toxicology Branch is particularly affected by this. Currently two drugs, dideoxycytidine (NSC 606170) and dideoxyadenosine (NSC 98700) are scheduled for INDA submission. Toxicity testing as well as continuous infusion studies of these drugs are in progress. New toxicology protocols for anti-AIDS drugs are being developed and toxicology test procedures, including analytical, acute and chronic toxicity and pharmacokinetics have been established.

b. Pharmaceutical Resources Branch (PRB)

The primary objectives of the Pharmaceutical Resources Branch are to supply high quality chemical substances and formulated products for investigative program use. Major tasks through which these objectives are realized include large-scale chemical preparation, chemical analysis, pharmaceutical development and manufacture, and storage, distribution and shelf-life surveillance.

During this year, over 100 compounds were prepared in an average quantity of 2.5 Kg. Such bulk preparations were completed for all compounds that passed DN-2A, including L-histidinol (NSC 74518), HMBA (NSC 95580), dideoxycytidine (NSC 606170), merbarone (NSC 336628) and BSO (NSC 326231). Detailed chemical analyses of all of these preparations was carried out. In addition, analytical methods were developed for a number of drugs in plasma and growth media.

New formulations and dosage forms were completed for 12 drugs, including clomesone (NSC 338947), BSO (NSC 326231), perilla ketone (NSC 348407) and triazine antifol (NSC 127755). Compounds exhibiting significant stability or solubility problems

were handled whenever possible by means of parenteral oil-water emulsions.

The PRB supported the supply and distribution of the various biological response modifiers that entered clinical trials. These included leuprolide, tumor necrosis factor, photofrin, zolodex and several monoclonal antibodies.

Like other Branches, the PRB has devoted a considerable effort to the development of anti-AIDS drugs. In particular, clinical supplies of suramin (NSC 34936) and azidothymidine (NSC 602670) have been prepared and maintained.

In order to comply with new Federal Regulations (OSHA) and State Laws the PRB has, in collaboration with the Toxicology Branch, other NCI Divisions, and the NIH Division of Safety, begun the preparation of Material Safety Data Sheets on the drugs provided to NCI contractors.

c. Office of Extramural Resource Analysis and Development (OERAD)

This newly-formed Office has the responsibility to provide an administrative and managerial focus for all DTP-supported external activities. These activities encompass contracts, grants and cooperative agreements.

Requests for Proposals were issued for 40 contracts during the year, and contracts have now been awarded in most of these procurements. Such contracts support the basic DTP effort and cover all tasks ranging from compound acquisition through storage and distribution of compounds, and biological testing to toxicology, pharmacology, computer support and clinical formulation. The total value of the 40 multi-year contracts is $102,695,543.

A total of 291 grants, with a dollar value of $43,834,000, have been awarded. These include traditional R01 grants, new investigator grants (R23), small business innovative research grants (R43/R44), academic research and enhancement grants (R15), but not Conference awards (R13).

Four National Cooperative Drug Discovery Groups (NCDDG) for the discovery of anti-cancer agents are being supported under Cooperative Agreements. These four groups involve 21 laboratories in 14 institutes and have a total FY86 value of $2,223,384.

A Request for Applications for an NCDDG for treatment of AIDS has been issued and twenty applications comprising over 70 laboratory programs have been received. These are currently in review and completion of this process is expected during June, 1986.

B. Intramural Program

1. Laboratory of Biological Chemistry (LBC)

The Laboratory of Biological Chemistry was established in 1985 to identify as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. An important aspect of this mission is to develop appropriate *in vivo* systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler *in vitro* model systems. Accordingly, the Laboratory is involved in the identification of endogenous factors present *in vivo* that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. Approximately half of the Laboratory's resources are applied to non-traditional targets including early key biochemical events signaling cell proliferation or differentiation. The other half of Laboratory resources is applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or over-expression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. Projects have therefore been initiated to develop new chemotherapeutic agents that block the action of growth factors. Non-traditional targets selected for drug design and study include: the second messengers inositol triphosphate and diacylglycerol; myristoylation of cellular oncogene products; protein kinase C; and the *ras* oncogene product.

Inducers of protein kinase C activity such as diacylglycerols and phorbol esters, have shown the importance of this enzyme in the regulation of growth factor activity, lymphoblastogenesis, normal cell proliferation, cellular differentiation and oncogene expression. A new investigation has been initiated to study the regulation of plasma membrane-associated tyrosine kinase in HL-60 leukemia cells undergoing differentiation and in cells resistant to differentiating agents. In addition, the relationship between membrane tyrosine kinase and pleiotropic drug resistance in the human breast carcinoma cell line MCF-7 is being investigated to assess the association between the p170 glycoprotein induced in multidrug resistant cells, calcium channels and tyrosine kinase. To investigate these problems, a new nondenaturing gel electrophoretic assay for multiple tyrosine kinase activities in cell extracts has been developed.

A new project has been initiated to determine the function of the ras oncogene product as a potential target for drug design. Activation of the ras oncogene has been implicated as the causative agent in as many as 30% of all human tumors. A recently characterized membrane protein, ARF, shares many features with the ras oncogene product, including size, location and ability to bind ATP. ARF was isolated from brain membranes and two of its amino acid sequences characterized and synthesized. From these fragments, oligonucleotide probes were synthesized and are being used to screen a cDNA library to obtain full length sequences of ARF. Antibodies to the sequences have also been made. These antibodies and cDNA probes will be used to identify the size and physiological role of the protein in the membrane.

Multidrug-resistance is a well documented phenomenon that limits the chemotherapeutic effectiveness of many traditional antitumor agents. During the past year an important advance was made in this Laboratory in the form of a tool to study the biology of the multidrug-resistant membrane protein(s). Photoactive radioactive analogues of vinblastine were used to photo-affinity label membranes of Chinese hamster drug-sensitive (DC-3F), multidrug-resistant sublines selected for resistance to vincristine (DC-3F/VCRd-5L) or actinomycin D (DC-3F/ADX), and revertant (DC-3F/ADX-U) cells. A radiolabeled doublet (150-180 kDa) consisting of a major and minor band which was barely detectable in parental drug-sensitive cells was increased up to 150-fold in the drug-resistant variants but only 15-fold in the revertant cells. Photoaffinity labeling in the presence of 200-fold excess vinblastine reduced radiolabeling of the 150-180 kDa species up to 96% confirming its Vinca alkaloid binding specificity. The radiolabeled doublet co-migrated with a polypeptide doublet in the drug-resistant cells and was immunoprecipitated with polyclonal antibody specific for the 150-180 kDa surface membrane glycoprotein (gp150-180) in multidrug-resistant cell lines. The identification of this Vinca alkaloid acceptor in multidrug-resistant plasma cell membranes suggests the possibility of a direct functional role for gp150-180 in the development of multi-drug resistance. These photoactive analogues will also serve as useful tools for the isolation of the gp150-180 proteins.

2. Laboratory of Experimental Therapeutics and Metabolism (LETM)

The LETM encompasses an interdisciplinary research program in tumor biology, pathology, biochemistry, pharmacology and toxicology that includes 1) utilization of the biochemical and pathobiological characteristics of hepatic and extrahepatic tumor cell populations to define the histogenesis of specific cancers; 2) establishment of specific animal and human tumor cell lines to study new and existting anticancer drugs for their mechanisms of toxicity; 3) investigation of biochemical and molecular mechanisms of drug metabolism and drug toxicity; 4) elucidation of the metabolic and physiological factors that may underlie target organ and target cell specific toxicity and prevention of toxicity by cellular defense mechanisms;

and 5) the continuation of the development of suitable cellular and animal models to elucidate mechanisms of toxicity of anticancer drugs and chemical modulators of anticancer drug effects.

The major emphasis during this report period has been on the characterization and establishment of human lung cancer cell lines for research on cell type specific chemical/drug interactions. A comprehensive program has been initiated to characterize lung tumors based on their morphological, ultrastructural, biochemical, metabolic and molecular genetic characteristics, as well as to establish *in vitro* and *in vivo* techniques for screening potential drugs against primary human lung tumors. Of particular interest during the current year has been the development of methods to propagate human lung tumors in the lungs of nude mice by a novel intrabronchial implantation method which will allow for improved *in vivo* propagation and drug screening studies.

In allied studies, a project has been initiated to study the metabolism of prostaglandins and related eicosanoids in human lung tumor lines, the objectives being to discover new targets for drug manipulation, and to determine if eicosanoid levels can be used to diagnose specific tumor types.

The development of methods in the LETM for the simultaneous qualitative and quantitative analysis of the five principal prostaglandins and their metabolites provides the analytical basis for determination of the role of this group of highly active compounds in the pathophysiology of human lung cancer and other human malignancies. Our observation that prostaglandin E_2 is the most prominent prostanoid biosynthesized by two human lung tumor lines (NCI-H322 and NCI-H358) in response to exogenous stimuli suggest that prostanoid biosynthesis may be a unique biochemical characteristic of a group or subclass of human primary lung carcinomas. Studies relating prostaglandin biosynthetic capabilities of human primary lung carcinomas *in vivo* and *in vitro* have been initiated. The *in vitro* studies of prostanoid production employ fresh human lung carcinoma isolates and the primary and early passage cultured cells derived from human lung carcinoma isolates. *In vivo* production of prostanoids, particularly prostaglandin E_2, will be determined via the urinary excretion rate of the major urinary metabolite of PGE_2 (7α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid; PGE-M) via high resoulution capillary gas chromatography-negative ion chemical ionization mass spectrometry.

3. Laboratory of Medicinal Chemistry (LMC)

In FY 1986, the former Laboratory of Pharmacology and Experimental Therapeutics (LPET) was divided into two components, the Laboratory of Biochemical Pharmacology (LBP) and the Laboratory of Medicinal Chemistry (LMC). The latter was established in order to give increased intramural emphasis to (1) the discovery and identification of new anticancer and antiviral drugs from both synthetic and natural sources and (2) the development of analytical methodology appropriate for the quantitation of new drugs in biological fluids

and the identification of metabolites. Essentially all projects are collaborative in nature, either among the synthetic and analytical chemists within the Laboratory or between the LMC and the laboratories within the DTP, the NCI or other NIH Institutes.

The discovery by Broder and Mitsuya that 2',3'-dideoxynucleosides (DDO-nucleosides) have anti-HTLV-III activity prompted the LMC to devote a large portion of its FY-86 resources to investigating this area of drug therapy. DDO-cytidine will be the first of these potential anti-AIDS compounds to enter Phase I clinical trial. The LMC analytical chemistry group developed a rapid, simple and sensitive HPLC method for quantitating DDO-cytidine in biological fluids suitable for both pre-clinical and clinical studies. This method has a limit of quantitation of 40 ng/mL. Using this method, extensive pharmacokinetic studies have been carried out in mice and monkeys. The compound is rapidly absorbed from the gastrointestinal tract, and thus can be administered by either parenteral or oral routes.

Other dideoxynucleosides have been synthesized. The 5-fluoro analogue of DDO-cytidine is as active against HTLV-III as DDO-cytidine, although 5-bromo and 5-methyl were inactive. The dideoxy analogue of 5-azacytidine was active but was also cytotoxic. The dideoxy analogues in the cyclopentenyl (CPE) series (i.e. analogues of neplanocin and of CPE-cytosine) were inactive, probably because of lack of substrate activity for deoxycytidine kinase and other nucleoside kinases.

A major objective of the Laboratory was realized when the cyclopentenyl nucleoside 3-deazaneplanocin was synthesized. The compound is a highly effective inhibitor of 5-adenosylhomocysteine (SAH) hydrolase and is a potent inhibitor of a number of viruses (although not of HTLV-III). With a K_i of 50 picomolar, this compound is 100 times more potent than the best inhibitor of SAH hydrolase previously known.

In a related synthetic area, the pyrimidine nucleoside analogues CPE-cytosine and CPE-uracil have been studied further. CPE-cytosine is phosphorylated by uridine-cytidine kinase, and (as the triphosphate) is a potent inhibitor of uridine-cytidine kinase, and thus offers promise as an inhibitor of the pyrimidine nucleoside salvage pathway.

4. Laboratory of Biochemical Pharmacology (LBP)

The Laboratory of Biochemical Pharmacology was established in January, 1986, by the division of the former Laboratory of Pharmacology and Experimental Therapeutics into two components which are concerned respectively with the design and synthesis of antitumor drugs (Laboratory of Medicinal Chemistry) and the mode of action of new antitumor drugs (Laboratory of Biochemical Pharmacology). The Laboratory studies new agents which have originated within the Developmental Therapeutics Program and

also agents derived from extramural sources in whose preclinical development the Program is playing a major role. In the last year, the Laboratory has also participated actively in elucidation of the cellular pharmacology of compounds with anti-HTLV-III activity currently under development within the Program.

In continuing studies with arabinosyl-5-azacytosine (ara-AC, an antitumor agent developed in this laboratory and now entering clinical trial), mechanisms by which tumor cells become resistant to the drug have been further elucidated. In all the murine systems studied to date, resistance to ara-AC has been accompanied by a fall, and, in some cases, a deletion of deoxycytidine kinase, the enzyme responsible for initiating the anabolic phosphorylation of the drug. In human tumor cell systems, a positive correlation has been established between sensitivity to the drug and deoxycytidine kinase levels. It is proposed that measurement of the kinase may be of prognostic value in patients receiving ara-AC in Phase I/II clinical trials.

Mitsuya and Broder have shown that 2',3'-dideoxynucleoside analogues of physiological purines and pyrimidines (2',3'-dideoxy cytidine, adenosine, guanosine and thymidine) are effective inhibitors of the replication of HTLV-III/LAV virus, probably by a viral DNA chain-terminating mechanism. The most potent of these agents, 2',3'-dideoxycytidine (DDO-cytidine), is currently under development for clinical trial. In studies of the cellular pharmacology of this agent in activated human T-lymphocytes in culture (ATH8 cells), we have demonstrated the formation of the mono, di- and triphosphates of DDO-cytidine. The initial phosphorylation step is catalyzed by deoxycytidine kinase, and does not occur in do exycytidine kinase-deficient cell lines. The physiological substrate 2'-deoxycytidine will effectively block the antiviral activity of DDO-cytidine by competitively inhibiting anabolism of the analogue. Apart from anabolic phosphorylation, DDO-cytidine is metabolically rather inert; slow deamination (to 2',3'-dideoxyuridine) is detectable in the monkey but not in other species examined to date.

Further progress has been made in the exploitation of our earlier observations of qualitative differences between the amino acid transport systems of tumor cells and host bone marrow cells, with tumor cells utilizing System L (the sodium-independent leucine-preferring amino acid transport system) while bone marrow progenitor cells do not. The objective has been the design and synthesis of new amino acid nitrogen mustards which are transported solely by System L and thus exhibit antitumor activity without myelosuppression. The most effective agent to date is 2-amino-7-bis(2-chloroethyl) amino-1,2,3,4-tetrahydro-2-naphthoic acid, the most potent competitive substrate-inhibitor of System L (K_i = 0.2 M) yet described, with a 500-fold greater affinity for this transport system than phenylalanine mustard (L-PAM). Comparative evaluation of the antitumor and myelosuppressive activities of L-PAM and the System L-specific nitrogen mustard indicates that the latter possesses both enhanced antitumor and reduced myelosuppressive

properties. Synthesis of a larger amount of the naphthoic acid mustard has now been carried out, in order to permit its study in a wider range of experimental human and murine tumor systems.

5. Laboratory of Molecular Pharmacology (LMP)

The Laboratory of Molecular Pharmacology during the current year has carried out studies in four major areas: (1) studies of the molecular mechanisms of action of alkylating agents, including chloroethylnitrosourea-like compounds and nitrogen mustards; (2) studies of topoisomerase II as a target of action of DNA intercalating agents and epipodophyllotoxins; (3) studies of the effects of incorporation of base analogs into cell DNA on DNA structural integrity as measured by DNA filter elution techniques; and (4) studies of the regulation of chromosomal protein biosynthesis in relation to the control of cell proliferation. A major goal is to develop strategies for the selective killing of particular human tumor cell types.

A simple and sensitive assay has been developed for the DNA repair enzyme guanine-O6-alkyltransferase (GO6AT). This enzyme can remove chloroethyl groups added to the 6-position of DNA guanine by alkylating agents such as the chloroethylnitrosoureas. In the absence of GO6AT (i.e., the Mer^-phenotype), the O6-chloroethylguanines in DNA can react further to form lethal interstrand crosslinks. There is thus a potential direct correlation between the GO6AT level of a tumor and its sensitivity to chloroethylating agents. The assay employs a synthetic double-stranded deoxyoligonucleotide which was designed so as to incorporate an O6-methylguanine residue in a sequence recognized by a restriction enzyme. While the methyl group remains in place, the restriction enzyme does not recognize the site and does not cleave the site. When the oligonucleotide is incubated with a cell or tissue extract that contains GO6AT activity, some of the methyl groups are removed, generating sites that the restriction enzyme can cleave. Cleaved strands (which bear a ^{32}P label at one end) are then quantitated. The present assay has successfully identified Mer^+ and Mer^- phenotypes in human tumor cell strains and in xenograft tumors and is applicable to clinical tumor samples.

Previous work in this Laboratory, using DNA filter elution techniques developed here, had shown that several DNA intercalating anticancer drugs (e.g. amsacrine, anthracyclines and ellipticines) produce DNA strand breaks and DNA-protein crosslinks in mammalian cells. The pattern of these effects indicates that DNA strands are cleaved and that at the same time a protein becomes covalently linked to the DNA at the cleavage sites. We refer to this type of DNA lesion as protein-associated strand breaks (PASB) and propose that these effects are due to drug action on one or more DNA topoisomerases. Work in several laboratories has confirmed the observations and has extended the list of drugs that produce PASB. In addition to new intercalating agents, such as mitoxantrone, the list of drugs that affect topoisomerases now includes the epipodophyllotoxins and camptothecin. During the past year, the identi-

fication of this activity as topoisomerase II was completed. As a result of this work, there is now a firm foundation for the use of DNA filter elution assays for the determination of topoisomerase alterations in intact cells.

Earlier studies in this Laboratory had disclosed differences between the set of histone variant proteins that are synthesized in quiescent (GO) cells, G1 cells and S phase cells. Work completed during the current year investigated effects of various DNA synthesis inhibitors and DNA damaging antineoplastic agents. A variety of effects were observed on histone variant synthesis patterns and a model to explain these effects was proposed. Work is under way to isolate a gene for a histone variant protein (H2A.Z) that is regulated characteristically in GO cells. Analysis of the DNA sequences that regulate the transcription of this gene may help to elucidate the control mechanism for cell proliferation at the genomic level. A number of clones from a human genomic library in charon 28 lambda were selected on the basis of hybridization to two oligonucleotide probe sets which were synthesized based on the amino acid sequence of the unique portion of the H2A.Z protein. In order to proceed further, it was necessary to test the candidate clones against mRNA species that are present in cells at very low levels. This required a new method for mRNA isolation, which has now been developed. The new method allows the simple and rapid isolation of small quantities of RNA from whole cells or cytolasmic extracts. The method should find wide application in molecular cell biology.

6. Laboratory of Tumor Cell Biology (LTCB)

While the overall objectives of the LTCB are to develop, implement, and analyze data obtained from studies of cellular proliferation, cell differentiation, and biochemical growth characteristics of normal and malignant mammalian cells both *in vivo* and *in vitro*, the major activities during this reporting period have focused on studies relating to the proposed etiologic agent of AIDS, namely HTLV-III. Among some of the many accomplishments by the laboratory are:

a. The demonstration that HTLV-III can replicate in human monocytes/macrophages as well as in T-lymphocytes. It has been suspected for some time that other cell types, in addition to T-lymphocytes, could act as a host for HTLV-III replication. This Laboratory has now established the presence of the virus in human monocytes/macrophages and has characterized the biological effects of HTLV-III on these cells.

b. Further characterization of the HTLV-III genome, including the localization of the tat gene and the demonstration that the tat gene is essential for HTLV-III function. The nucleotide sequence of the envelope gene of five new isolates of HTLV-III has been determined, and conserved and divergent regions have been localized and identified. The protein products of the tat-III, sor and 3'orf genes have been found to be expressed *in vivo* and to be immunogenic.

c. Further progress has been made on the development of second-generation antibody tests for HTLV-III and of procedures for standardization of HTLV-III serology.

d. Efforts have continued on the detection and development of agents to inhibit the replication and infectivity of HTLV-III. It has been found that synthetic oligonucleotides complementary to the tat-III gene splice acceptor and donor sites can act as potent inhibitors of HTLV-III replication in cell culture.

e. Studies on neutralizing antibodies to HTLV-III have continued. Inactivation of HTLV-III by antibody to thymosin α-1 has been demonstrated in cell cultures, indicating that antibody to thymosin α-1 is a neutralizing antibody. Thymosin α-a has been found to be homologous to HTLV-III p17.

Table 1

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/85 - 3/31/86)

NSC Number	Name	Compound Type*
	Decision Network 2A	
369327	Elsamicin	NP
374610D	Discreet	S
374613D	Discreet	S
	Decision Network 2B	
326231	L-Buthionine Sulfoximine (BSO)	S
328426	Phyllanthoside	NP
339004	Chloroquinoxaline Sulphonamide	S
363812	Tetraplatin	S
368390	Biphenquinate (DUP-785)	S
	Decision Network 3	
336628	Merbarone	S
347512	Flavone Acetic Acid	S
356894	Deoxyspergualin	NP
368390	Biphenquinate (DUP-785)	S

Table 1 (con't)

COMPOUNDS THAT PASSED DECISION NETWORK (4/1/85 - 3/31/86)

NSC Number	Name	Compound Type*
	Decision Network 4	
125973	Taxol	NP
172112	Spiromustine	S
253272	Caracemide	S
352122	Trimetrexate	S
	Compounds in DN Special	
74518	L-Histidinol	S
98700	Dideoxyadenosine	S
348407	Perilla Ketone	NP
373965D	Discreet	S
403169	Acronycine	NP
606170	Dideoxycytidine	S
606874D	Discreet	NP

*S = synthetic
NP = natural product
SS = semi-synthetic (natural product modified synthetically)

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 - September 30, 1986

The fundamental objective of the Drug Synthesis and Chemistry Branch (DS&CB) is the discovery and development of novel synthetic anticancer leads through the acquisition , synthesis and management of the flow of unique synthetic compounds for evaluation as potential anticancer agents in the Developmental Therapeutics Program (DTP).

The DS&CB achieves its central mission by engaging in a variety of Program activities, namely, acquisition of a large number of diverse synthetic compounds on a worldwide basis, pre-selection development, synthesis of congeners and prodrugs, radiolabelled syntheses, task order resyntheses, storage, inventory and distribution and computer-assisted structure-activity analysis. In addition, DS&CB supports the activities of the Information Technology Branch (ITB), the Radiation Research Program (RRP), the European Organization for Research on Treatment of Cancer (EORTC) Program and the U. S. Japan Cooperative Cancer Research Program. Collaborative programs have been established with the intramural laboratories; the Laboratory of Biological Chemistry for the design and synthesis of inhibitors of phospholipid metabolism and the Laboratory of Medicinal Chemistry for the synthesis of antiviral nucleosides.

The contracts managed by DS&CB are outlined in Table 1. Presently, the DS&CB is staffed with five professionals and three clerical personnel.

The major challenge during this year has been to anticipate the needs of our evolving "disease-oriented" program and restructure the program activities of DS&CB and the staff to respond in a creative and timely fashion to the new opportunities.

The accomplishments of DS&CB include the following:

1) The planned phasing-out of the input of new compounds to the P388 pre-screen from 10,000 to zero.

2) The design and synthesis of a soluble tetrazolium derivative as a reagent for the "in vitro" cell line test system.

3) The identification of several interesting groups of compounds for future screening in the "disease-oriented" in vitro cell line system; both from our own inventory as well as industrial suppliers like Merck and Warner-Lambert/Parke Davis.

4) Flavone acetic acid (NSC-347512), a compound previously acquired from Lipha continues to be of high interest in view of its exceptional activity against Colon 38. This finding has stimulated the interest of other research organizations to participate in our program.

5) Both Tetraplatin (NSC-363812) and diazohydroxide (NSC-361456), are progressing well in our program. Our patent application on Tetraplatin has been allowed and licensing negotiations are underway to ensure its development to the clinic. Two other compounds are in early stages of synthesis and development, a water-soluble taxol derivative, NSC-600221, and a new imidazolyl biscarbamate, NSC-371417 active in the LOX tumor.

6) The collaborative program with the Laboratory of Biological Chemistry has yielded ten cyclitol derivatives for evaluation as inhibitors of phospholipid metabolism.

7) The synthesis of several radiolabelled compounds for preclinical and clinical studies were completed, for example, flavone acetic acid (NSC-347512), ipomeanol (NSC-349438), merbarone (NSC-336628), and dideoxycytidine (NSC-606170).

8) The ten task order resynthesis contracts have provided a variety of compounds of program interest that are not available in sufficient quantity from the original suppliers (approximately 150 compounds) in a cost-effective way. These compounds support several segments of DCT programs including our current screens, intramural laboratories, AIDS' projects, Radiotherapy Program and the new in vitro screening system.

9) The storage, inventory and distribution contractor has shipped more than 16,000 compounds to contract screeners, formulation laboratories, NCI and NIH investigators in 45 states of the U. S. and 30 foreign countries. A major additional undertaking under this contract has been the processing and reshelving of approximately 16,000 compounds that were returned by the screening laboratories. These compounds will become important as the screening capacity of our new disease-oriented test system grows.

10) A concerted effort was undertaken to realign the DS&CB program and the staff to the new "disease-oriented" approach for anticancer drug discovery and phase out the P388-centered approach.

TABLE I

CONTRACTS - FY 86

Contractor	Investigator	Contract No.
Alabama, University of	Baker	NO1-CM-27571
Flow Laboratories, Inc.	Wilks	NO1-CM-27505
Georgia Institute of Technology	Zalkow	NO1-CM-27517
Japanese Foundation for Cancer Research	Sakurai	NO1-CM-36011
Maxima Corporation	Sobers	NO1-CM-47628
Research Triangle Institute	Kepler	NO1-CM-67703
SRI International	Lee	NO1-CM-47611
Starks, C. P., Inc.	Schultz	NO1-CM-87206
State University of New York, Research Foundation	Anderson	NO1-CM-27570

Master Agreements (Task Order) Contracts:

Alabama, University of	Baker	NO1-CM-37631
Eagle-Picher Industries, Inc.	Roth	NO1-CM-37634
Raylo Chemicals, Ltd.	Muhs	NO1-CM-37635
Research Triangle Institute	Seltzman	NO1-CM-37636
SISA, Inc.	Razdan	NO1-CM-37637
Southern Research Institute	Montgomery	NO1-CM-37638
South Florida, University of	Owen	NO1-CM-37639
Southwest Research Institute	Lyle	NO1-CM-37640
SRI International	Smith	NO1-CM-37641
Starks Associates	Starks	NO1-CM-37642

PUBLICATIONS:

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Haugwitz, R. D., Martinez, A. J., Venslavsky, J., Angel, R. G., Maurer, B. V., Jacobs, G. A., Narayanan, V. L., Cruthers, L. R., and Szanto, J. Antiparasitic Agents. 6. Synthesis and anthelmintic activities of novel isothiocyanatophenyl-1,2,4-oxadiazoles. J. Med. Chem. 28: 1234-1241, 1985.

Narayanan, V. L., Paull, K. D., Nasr, M. Computer-assisted Structure Activity Analysis of Barbiturates and their Thio-isosters and the Antitumor Activity of the Novel Experimental Antitumor Agent 5-(N-phenylcarboxamido)-2-thiobarbituric acid, NSC-336628. In J. Ishigami (Ed.): Recent Advances in Chemotherapy-Anti-cancer Section. Univ. Tokyo Press, 1985, 487-488.

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Nasr, M., Paull, K. D. and Narayanan, V. L.: Computer-assisted structure-anti-cancer activity. Correlations of carbamates and thiocarbamates. J. Pharm. Sci. 74: 831-836, 1985.

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Wolpert-DeFilippes, M. K., Anderson, W. K., Quagliato, D. A., Haugwitz, R. D., Narayanan, V. L.: Synthesis, Physical Properties and Antitumor Activity of Platinum (IV) Complexes of 1,2-diaminocyclohexane. Cancer Treat. Rep. In Press

Deutsch, H. M., Gelbaum, L. T., McLaughlin, M., Fleischmann, T., Haugwitz, R. D., Zalkow, L. H.: Synthesis of Congeners and Prodrugs of the Benzene Maleimide Photoadduct, Mitindomide as Potential Antitumor Agents. J. Med. Chem. In Press

ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 - September 30, 1986

The Natural Products Branch is responsible for acquisition, isolation, structure determination, and testing of compounds from microbial, plant and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch are: (1) acquisition of crude biological materials of plant, marine, and microbial origin for the DTP screening program; (2) contract research directed toward isolation of new agents from active extracts; (3) world-wide literature surveillance, and acquisition of natural products with demonstrated biological activity or novel structural types for evaluation; (4) procurement and preparation of large quantities of active agents for drug formulation, tumor panel testing, toxicology and clinical studies.

With the commitment of the Developmental Therapeutics Program to a new direction in in vitro screening, a renaissance of interest in natural products as potentially highly selective antitumor agents has begun with a major new program effort in collection of natural products from a wide variety of sources, including terrestrial plants, marine invertebrates, algae and marine microorganisms. New contracts have been awarded in FY '86 for collection of plants from diverse locations, collections of both shallow water and deep water marine organisms, and for cultivation of cyanobacteria.

Organization and Staffing

The Branch is organized into three functional segments coordinated through the Chief which are (1) acquisition of new pure compounds and supplier liaison, (2) isolation of new agents from fermentation derived extracts, and (3) isolation of new agents from plant and marine animal extracts. Many tasks require interaction between these segments, and the Branch personnel are assigned duties in whichever of the areas requires their expertise depending on changing program needs. The present full time staff consists of four professionals and two secretaries. The contracts managed by the Branch are outlined in Table 1.

Table 1.

Natural Products Branch Contracts

Contractor	Investigator	Contract/RFP No.	Program Area
Bristol Myers	Claridge	NO1-CM3-7556	Fermentation
Microbial Chemistry Research Foundation	Umezawa	NO1-CM4-7593	Fermentation
Polysciences, Inc.	Sims	NO1-CM5-7707	Plant Scale-Up

Univ. of Illinois	Farnsworth	NO1-CM6-7705	Literature Surveillance
Univ. of Connecticut	Pearce	NO1-CM5-7692	Fungal Fermentation
Warner-Lambert	French	NO1-CM3-7614	Fermentation
(In competition)		NCI-CM5-7740	Deep Water Marine Collection
(In competition)		NCI-CM5-7741	Shallow Water Marine Collection
(In competition)		NCI-CM5-7746C	Plant Collection (Asia)
(In competition)		NCI-CM5-7746A	Plant Collection (Africa)
(In competition)		NCI-CM5-7746B	Plant Collection (S. America)
(In competition)		NCI-CM5-7745	Cultivation of Blue-Green Aglae

Fermentation

The objective is to discover, isolate and develop novel antineoplastic drugs derived from microbial sources. There are five contracts to support this task although three have been terminated this year. Three contracts are devoted to the isolation of unique organisms and the systematic evaluation of microorganisms for their ability to produce novel anticancer agents. The contracts include selection and screening of microbes, fermentation, fermentation development, genetic and culture research, chemical isolation, identification and scale-up production of active materials of interest for NCI evaluation.

One contract which was awarded this year is devoted to the isolation and fermentation of various genera of fungi to provide NCI with cell extracts and lyophilized broths for evaluation for antitumor activity.

The PRI-FCRF contract continued to be a service contract primarily for pilot plant research and development and for the production of active agents in quantities sufficient for toxicology and clinical trials.

A new program in the cultivation of blue-green algae has been developed and the contract will be awarded.

Plant and Animal Products

The major objective of this program, namely the acquisition of novel antineoplastic agents from higher plants and animal sources (primarily marine), has mainly been achieved by the provision of in vivo and in vitro screening services to NIH grantees and interested scientists worldwide; subsequent fractionation of active extracts and isolation of active constituents has been aided by in vitro bioassay services. The award, by the end of FY '86, of contracts for extensive collec-

tions of shallow- and deep-water marine organisms, and plants from tropical rain forest regions, will greatly expand the scope of this acquisition program. Plans for the establishment of a facility for the extraction of these organisms and storage of the extracts under contract to the NCI are currently in progress. Extracts will be tested in the new *in vitro* primary screen comprising human cancer cell lines, and organisms yielding active extracts will be re-collected in larger quantities and investigated as sources of novel antitumor agents, either by an in-house isolation group to be established at a later stage, or by external research groups working in collaboration with the NCI. In the case of pure compounds exhibiting promising selective activity in the NCI screens, larger amounts will either be purchased from the investigator or produced under a contract maintained for such scaleup isolations. Large quantities of plant-derived drugs needed for preclinical and clinical studies, including phyllanthoside and taxol, have been produced by such a contract over the past year.

Worldwide Surveillance of Natural Products

This function is primarily carried out in the Office of the Chief. The objective is to acquire new natural products with biological activities which may relate to anticancer effects, and compounds of a wide variety of new or unusual structural types which are worthy of screening for antitumor activity. The approach to identification of compounds for acquisition is multifaceted, and includes a contract for literature surveillance which identifies new natural product structures and biological activities, literature review by Branch staff, personal contacts with scientists in universities, research institutes and chemical and pharmaceutical companies, attendance at scientific meetings where new compounds are reported, and review of progress reports of NIH grantees. During calendar year 1985 a total of 476 new pure natural products were acquired, of which 375 (79%) were donated and the remaining 101 compounds (21%) were from contractors and grantees.

The literature surveillance program continues to be successful in bringing in new materials for screening. In 1985 257 compounds (54% of new acquisitions) were received in direct response to requests for those specific compounds, while a substantial number of unsolicited compounds received have come from suppliers who were contacted by our program previously in regard to other compounds. The response rate to our literature requests is 32% with 25% of responders supplying the requested compounds and another 7% sending regret letters or supplying other compounds or making inquiries about the program.

Additional details are found in Table 2 which shows that foreign suppliers are currently the source of 51% of the input. The number of compounds acquired for screening is up 28% over last year.

Table 2.

Pure Compounds Acquired in Calendar Year 1985

Compounds by Type and Source

	Industry	Univ.	Res. Inst.	Other	Domestic	Foreign
Plant	22 (8%)	195 (74%)	44 (17%)	2 (1%)	121 (46%)	139 (53%)
Fermentation	27 (40%)	28 (42%)	12 (18%)	0	18 (27%)	48 (73%)
Animal	41 (60%)	27 (40%)	0	0	19 (28%)	49 (72%)
Synthetic	0	82 (100%)	0	0	77 (95%)	4 (5%)

Compounds by Type and Mechanism

	Grant	Contract	Lit. Sur.	Unsol.
Plant	75 (29%)	0	116 (45%)	69 (26%)
Fermentation	0	9 (13%)	27 (40%)	31 (46%)
Animal	17 (25%)	0	45 (67%)	5 (7%)
Synthetic	0	0	69 (84%)	13 (16%)

Accomplishments

A major new program in collection of natural products for screening, extraction and storage of extracts, and isolation of active constituents from these extracts has been developed, and initial contracts have been awarded, as a major initiative of the Division of Cancer Treatment. This new program will supplement the acquisition of new natural products through the literature surveillance project which has continued to play an indispensable role in the drug discovery program (Table 2).

Natural Products have continued to be an important source of compounds in preclinical development and early clinical studies as outlined in Table 3.

Collaborative programs are on-going with outside investigators to develop improved second generation drugs which are designed to obviate the problems with the parent molecules. One project is underway with Dr. Leon Zalkow of Georgia Institute of Technology and Dr. Garth Powis of the Mayo Clinic on analogs of indicine N-oxide designed to increase antitumor activity and decrease hepatotoxicity. A second project is being done in the area of camptothecin derivatives with Dr. Monroe Wall of the Research Triangle Institute which has resulted in discovery of several new compounds with increased activity over the parent compound, and with Dr. Ahmed Ahmed of the University of Texas in camptothecin metabolism.

Deoxyspergualin, a novel polyamine derivative with powerful antileukemic activity in mice, has entered Phase I clinical trials.

Major presentations on the new screening program have been given at conferences on marine natural products chemistry, organic chemistry and pharmacognosy and have resulted in a high level of interest from outside investigators.

A major effort has been made to contact Chinese scientists to establish collaborations in natural products screening and a lecture is planned at the First Beijing International Cancer Symposium.

An IND application is being prepared for Fostriecin and large quantities of the compound have been prepared for Phase I trials. Formulation and stability studies on Elactocin have been completed with large quantities of the compound prepared for further development.

Elsamicin has passed DN2A. Preclinical toxicology is currently being done at Bristol. Another highly potent compound is being formulated and pilot toxicological and pharmacokinetic studies are underway.

A scale-up fermentation from 30 to 300 gal tanks to produce a highly potent, wide spectrum antitumor antibiotic of potential clinical interest to NCI has been accomplished at FCRF under a collaborative agreement with Bristol Laboratories.

Table 3.

Natural Products in Advanced Development

NSC#	Drug	Origin	Status
163501	Acivicin	Fermentation	Phase II Clinical Trial
218321	Pentostatin	Fermentation	Phase II Clinical Trial
526417	Echinomycin	Fermentation	Phase II Clinical Trial
141633	Homoharringtonine	Plant	Phase II Clinical Trial
125973	Taxol	Plant	Phase II Clinical Trial
269148	Menogaril	Fermentation	Phase II Clinical Trial
325319	Didemnin B	Marine Animal	Phase I Clinical Trial
328426	Phyllanthoside	Plant	Toxicology
356894	Deoxyspergualin	Fermentation	Phase I Clinical Trial
303812	Aphidicolin Glycinate	Fermentation	Toxicology (With E.O.R.T.C.)
339638	CI-920	Fermentation	INDA Filing
332598	Rhizoxin	Fermentation	Activity Studies
364372	Elactocin	Fermentation	Toxicology
369327	Elsamicin*	Fermentation	Bulk Production

* Passed Decision Network 2A FY '86

Staff Publications

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2. Nassimbeni, L.R., Niven, M.L., Cragg, G.M., and Pettit, G.R.: The structure of phyllanthose hexaacetate, $C_{24}H_{34}O_{15}$. Acta Cryst. C41: 728-731, 1985.

3. Pettit, G.R., Cragg, G.M., and Suffness, M.: Phyllanthostatin 1-phyllanthoside orthoacid rearrangement. J. Org. Chem. 50: 5060-5063, 1985.

4. Pettit, G.R., Singh, S.B., and Cragg, G.M.: Synthesis of natural(-)combretastatin. J. Org. Chem. 50: 3404-3406, 1985.

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ANNUAL REPORT OF THE BIOLOGICAL TESTING BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The primary functions of the Biological Testing Branch (BTB) include responsibility for biological pre-screening, in vitro screening and selective follow-up testing of a large number of candidate cancer chemotherapy compounds to support the in vivo aspects of this program. The BTB is responsible for the production, quality control, and distribution of genetically and biologically defined rodents. The BTB manages a very large resource for the production of genetically-controlled and disease-free experimental animals. Because of its large size, unique quality control capabilities, and economy of production, the BTB is able to supply, in addition to DTP/DCT needs, those of other NCI Divisions, other institutes within the NIH, other governmental agencies, and NIH grantee investigators on a cost reimbursement basis.

The BTB maintains a large repository of experimental animal and human tumor lines for internal usage by DTP and other NCI programs. Tumors are also distributed to qualified cancer research investigators on a cost reimbursement basis.

The BTB is responsible for staffing and maintaining the DCT intramural Barrier Facility in Building 37.

Human tumor cell lines are acquired, characterized extensively and adapted for in vitro and in vivo testing purposes. In vivo models are developed and evaluated for appropriate follow-up testing of those agents which are found to be of interest in the in vitro cell line screening project.

The objectives of the BTB are to:

1. Enhance in vitro testing capabilities from the current capacity of approximately 40 human tumor lines to encompass more than 100 lines of diversified origin including lung, colon, mammary, renal, brain, ovarian, prostate, melanoma tumors, etc. which are well characterized and defined. The time frame for accomplishing this most important objective depends upon the allocation of additional interim space and the construction of permanent facilities at FCRF.

2. Initiate mechanistically-oriented antitumor drug pre-screens for use in conjunction with the in vitro cell line screening project described above. It is anticipated that this effort will be managed as a subcontract program at FCRF.

3. Make necessary adjustments in in vivo testing to the extent that it satisfactorily complements the in vitro screening program. These adjustments will include:

4. Continuing to provide laboratory animals of the quality (from both an animal health and genetic integrity view point) and quantity, to meet the needs of the various programs using these services with necessary adjustments in production capabilities.

5. Maintaining the Building 37 barrier facility as well as possible under existing circumstances.

Accomplishments:

1. During the past year substantial progress has been made on the In Vitro Cell Line Screening Project at FCRF. A large number of human tumor cell lines (>85) have been acquired and a subset selected for detailed characterization prior to inclusion in disease-oriented drug screening panels. Consistent with the initial emphasis of the project on lung cancer, and the complexity of the disease, the most extensive cell line panel developed thus far, is for lung cancer. Representatives of adenocarcinoma, adenosquamous, squamous cell, bronchioalveolar, large cell, mucoepidermoid, as well as "classic" and "variant" forms of small cell carcinoma have been characterized for use in large scale in vitro screening. More recently, cell lines have been acquired for development of disease-oriented panels for colon, renal, and ovarian carcinoma, as well as melanoma and CNS cancer. Several "miscellaneous" cell lines have also proven to be useful for in vitro screening. These include the murine P388 leukemia (long utilized as the in vivo pre-screen), MCF-7 breast carcinoma, WI-38 fetal lung fibroblasts, and multi-drug resistant variants of P388 and MCF-7 (P388/ADR and MCF-7/ADR).

   Protocols for automated growth inhibition assays with these cell lines have been developed and optimized. Substantial progress has also been made on a "second generation" colorimetric assay which utilizes a new tetrazolium reagent which was designed and synthesized for the project. The second generation assay reduces the number of steps from five to three and thus substantially streamlines the assay.

   Pilot-scale screening of unknown compounds was initiated in February, 1986. A pilot screening panel of ten non-small cell lung cancer cell lines and six "miscellaneous" cell lines was selected for initial use. One hundred and eight compounds were selected from the past ten years acquisitions using a random number generator. These were utilized to gain initial experience in screening and to test the performance of an automated data processing support system (IVSS) which was developed for the project. The results of this screening have been very encouraging. The majority of compounds showed very similar activity in all of the cell lines tested. A single compound showed a dramatically different pattern; differential cytotoxicity at very low doses. IC_{50} values (the concentration of test compound producing inhibition of growth to 50% of the control value) for this compound varied over more than a three log range. Thus, the results of this initial pilot-scale screening support the concept of screening for compounds which exert differential cytotoxicity and indicate that the assay methods developed are well suited to the task. Addition of cell line panels for additional tumor types will make screening for tumor selective drugs feasible.

2. A. Tentative protocols have been developed for subrenal capsule testing with 4 in vivo correlating human tumor models. An additional 6 sub-renal models are in the process of protocol development.

   B. The microencapsulation model is being evaluated for in vivo follow-up testing. Preliminary evaluation indicates that this model is likely to be cost and time effective for preliminary testing but may be difficult to quantitate cell kill.

   C. Intrabronchial implantation (IB)techniques have been employed with several human lung tumor lines from the in vitro screen. This model provides for implantation within the right mainstem bronchus of athymic mice via tracheostomy and subsequent inoculation of all suspensions through a specially modified intrabronchial catheter. This model offers advantages for in vivo propagation and testing of lung tumors with the prospective that quantitation of cell kill can be achieved.

   D. A practically useful metastatic model of human cancer has become available. Work at FCRF and Southern Research Institute with the human LOX melanoma has resulted in selection of cell lines with enhanced metastatic potential and development of a standardized protocol for use in preclinical evaluation of antitumor drug candidates. The protocol represents a bioassay approach which gives information regarding the activity of drugs against growing pulmonary metastases. Tumor fragments are implanted subcutaneously into groups of athymic mice. The mice are then held for approximately 30 days. During this time, tumor cells spontaneously metastasize to the lungs. Test compounds are then administered to animals (preferably by the i.v. route) and the following day animals are sacrificed and the lungs removed. Lung fragments are transplanted subcutaneously to bioassay animals. Drug effects are reflected in inhibition of growth of tumors from the transplanted lung fragments.

3. A payback system to recover costs of experimental tumor distribution to qualified investigators throughout the world was successfully implemented at FCRF.

4. As sufficient tumor material has become available, tentative protocols have been developed for four in vivo models for the disease-oriented screen. Three additional models are in final testing, three are undergoing preliminary studies. Other lines will undergo preliminary studies as soon as large scale cell production makes adequate material available. An increasing amount of test capacity was committed to development of these models.

   During the period 4-1-85 through 3-31-86, 7,671 new synthetics and 245 new natural products entered the P-388 pre-screen. Of these materials, 353 demonstrated confirmed activity. Current entry into this pre-screen has been reduced to the rate of 2,000 compounds per year.

   Approximately 60% of total in vivo test capacity was committed to tumor panel testing and further studies of drugs in development toward clinical trial. Of those compounds available in sufficient quantity for tumor panel testing, 87% have been completed.

5. Changing needs of animal users, including new strain requirements (primarily at FCRF), and adjustments in DTP *in vivo* screening needs (increased emphasis on athymic mice) have been handled expeditiously.

BIOLOGICAL TESTING BRANCH

FY 1986

PRIMARY GENETIC CENTERS (4)	$4,722,970
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
RODENT PRODUCTION CENTERS (4)	689,301
Large-scale production of inbred mice and nude mice under both conventional and barrier controlled environment.	
DIAGNOSTIC & HISTOCOMPATIBILITY PROJECTS (8)	997,289
To monitor animal health and genetic integrity.	
DEVELOPMENT OF STANDARDS & GUIDELINES (1)	35,500
For animal care and breeding.	
MAINTENANCE OF FROZEN TUMOR BANK (1)	370,000
FREDERICK CANCER RESEARCH FACILITY (2)	2,353,515
CENTRALIZED REDERIVATION (1)	148,474
Rederiving new starts from the NIH Repository into associated flora status.	
IN-VIVO SCREENING (4)	
Screening of potential anti-cancer drugs	2,654,469
COLONY FORMING ASSAY (3)	696,551
SCREENING QUALITY CONTROL (1)	518,389

FREDERICK CANCER RESEARCH FACILITY (2)

Animal Production	$2,053,515
Administrative Support	50,000
Shared Services/Renovations for APA	250,000
In-Vitro Cell Line Screen	1,000,000
Microencapsulation	85,716
Human Tumor Procurement and Preparation	289,076
	3,728,307

TOTAL	$14,561,250
Less Reimbursements (Including Grantee Collection)	2,986,250
NET COST TO DTP	11,575,000

ANNUAL REPORT OF THE INFORMATION TECHNOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Drug Information System has now been in use for over one year by DTP staff as well as scientists outside NCI and is working satisfactorily. Further efforts in this area are focusing upon natural products acquisition, pharmaceutically formulated materials and toxicology and pharmacology data. The ITB also continues to provide support to DTP in the area of animal production and use, application of personal computers to various problems, and the application of computer graphics in a number of areas where large amounts of data make data review difficult.

A major new effort for the ITB is the development of a computer system for use in the in-vitro screening effort at FCRF. An operating system has been installed and appears to support most of data acquisition and processing needs. Generation of a data retrieval system is the major remaining task in this area. Work has continued on the use of robotics in the in-vitro screening effort. The robot in DTP can weigh samples totally automatically and the loading of testplates by the robot is currently being investigated.

Office of the Chief, ITB

This Office supervises and coordinates the activities of the two Sections of this Branch, directs the progress of the DIS and initiates and pursues appropriate information activities with other units within NCI and NIH, other Agencies of the government, and private sector organizations.

1. Drug Information System.

This has been the first full year of operation of the Drug Information System (DIS). The central data retrieval capabilities of the system have been stable throughout the year, supporting some 2,000 sessions, or 10,000 transactions per month. There is no major change going on in this area. Development work has continued on other areas of the system. The automatic weighing capability used by the storage contractor has been re-structured to cope with a problem that resulted from poor response times. The use of the DIS by distant units, notably screening contractors, has been expanded and several minor telecommunications problems have been corrected. Working specifications for incorporation into the DIS of data pertaining to materials prepared under Good Manufacturing Practices were finalized and the implementation was completed.

Planning is now well advanced for the natural products acquisition and isolation effort. Raw data collection in the field is already supported (see below) and the framework for a computer system to support the inventorying and extraction of natural materials has been completed. This will allow the Natural Products Branch to acquire, store and extract natural materials, test the different extracts and register any active materials discovered in this way. The system is closely linked to the DIS in that specific active compounds isolated from natural materials will be merged immediately into the DIS.

At a more preliminary stage, plans have been made to provide data acquisition support for the pharmacology and toxicology efforts within DTP. The first goal in this work is to capture data from laboratories involved in pharmacology and toxicology and support DTP analysis of the data in an interactive computing environment.

During this year, the DIS has been made public to a select group of non-government scientists, who use it directly. Because such users may see non-confidential data and confidential data that they provided, an elaborate security system has been incorporated into the DIS which facilitates selective viewing of data according to the identity of the user. To date, 15 such users, from U.S. corporations and universities in the U.S. and Europe, have been provided with DIS access. A feature of the DIS is its ability to generate and print automatically letters requesting samples of compounds from suppliers. An enhancement has been added whereby these letters are now generated in the language most appropriate to the supplier. Languages used include French, German, Japanese and Spanish. Completion of this item marks the full assimilation of DTP's laser printer into the DIS environment. This printer generates documents containing any required mix of graphics and text, at the rate in excess of one page per second. Such pages can carry, as an example, a letter addressed in English, but written in Japanese and containing chemical structure diagrams imbedded in the (Kata-kana) text. The laser printer also serves as DTP's major means of producing output in hard copy. Some hundreds of structures are printed each week on this machine.

Programs which gather and report DIS usage statistics have been installed in the DIS and are now working. They run semi-automatically upon a monthly basis and provide ITB staff with a detailed analysis of DIS use. This is invaluable both to detect any security breach (there have been none) and to plan future enhancements to the system.

A series of six papers describing the entire DIS was prepared during January and submitted for publication.

Interest in graphics as a means of data representation continues to increase. All members of the Branch are now competent to some degree in computer graphics and graphical output of chemistry, biology and management data is now available to DTP staff for routine use.

2. Computer System for the Cell Line project.

A great deal of effort has gone into this project during the reporting period and an initial version of a data acquisition and processing system was released in October, 1985. After several weeks of use, a number of redesign parameters were defined and a second release was completed in April, 1986. This second release fulfills most of the requirements and is now undergoing touch-up modifications. The in-vitro tests are scheduled and prioritized in the IBM 370 then down-loaded to a micro-computer at FCRF. The micro-computer controls the inoculation and loading of plates, handles the plate reading, processes the raw data from the reading and finally uploads all the test data back to the mainframe. Meanwhile, a database structure for dealing with the large amounts of incoming data has been established on the mainframe and specifications have been developed for incorporation of these data into the DIS as

an independent database. Development of computer support for the cell line project has thus kept just ahead of the actual laboratory activities; it is not rate-determining nor has it moved so far ahead as to be out of touch.

Biological Information Section, BIS

1. Screening Database.

This year has proved to be a watershed year for the screening database. The amount of in-vivo testing has been sharply reduced; the pre-screen almost eliminated, and the entire database has been installed in the DIS. The DIS-resident database is essentially frozen, because few new data are being generated. Whatever new data appear are appended to the main file and an annual consolidation is envisaged. The database of in-vivo testing is thus searchable online. A variety of problems associated with searching such a large file have been encountered and most of them have been resolved or ameliorated.

2. Data Retrieval and Report Generation.

Efforts to reduce the repetitive generation of paper reports continue and this year, the "SAC Report" was finally abandoned. This report, issued every two weeks, detailed the status of all 14,000 Selected Active Compounds. The information it contained can be generated online for any or all of the 14,000 compounds and the routine printing and distribution of this report was no longer necessary. A variety of other reports have also been discontinued, so that the number of routinely produced reports now stands at 34, down from over 300 three years ago.

3. Microprocessors.

This Section first acquired a microcomputer in 1984 and has since explored its use in a variety of applications. During this reporting period, 15 additional microcomputers were acquired by DTP and ITB assisted in this acquisition and in the installation of the machines. As a result, microcomputers are now used by DTP for tasks ranging from data acquisition to word processing.

4. Animal Utilization.

The animal utilization reporting system that was developed by ITB last year is now in routine use and generally reliable accounting of mouse utilization is gathered on a regular basis. A parallel system dealing with the production and distribution of animals was purchased in early 1985 but was found to have numerous problems. In a combined effort between ITB and the commercial supplier, this system has been extensively revised and currently is operating fairly reliably. Some minor remaining problems are now being addressed.

5. Plant Collection Database.

Software to support the plant collection effort has been written and tested. It is intended to provide this software and a personal computer to field staff who will collect data on a floppy disk at plant collection time. The disks will be mailed to ITB where the data will be incorporated into the central database. Work in this area is complete and is waiting for the collection effort to begin.

6. Graphical representation of biological data.

When massive amounts of data have to be reviewed, as happens with the in-vivo testing data, computer graphics can be useful. A number of programs have been developed and made available to DTP staff for the generation of survival plots, inhibition plots and IC50 barplots for in-vitro testing data as well as budget plots, useful for management purposes.

Chemical Information Section, CIS

1. Chemical Structure Handling.

The DIS contains several programs for structure input and structure output and is now very strong in this difficult area. Structure entry programs written for the IBM PC run extremely rapidly; they appear to be faster than any other published programs. The printing of structures is also proceeding very smoothly. Structures, and most other graphics, can be imbedded into text and other materials and printed on the laser printer. In two related developments, about 1,000 structures in the DIS which had been entered incorrectly at CAS have been detected and corrected and a "beautification" program has been written and put into use. This takes any chemical structure and transforms it into a structure which meets established standards. Hexagons are normalized, alphanumeric characters are spaced properly and overwriting is obviated. Most importantly, the "dot notation", in which carbon atoms were denoted by an asterisk, is eliminated in favor of the modern vertex notation. All the structures in the DIS database, including newly entered structures can now be massaged by this program, which produces a very high final quality.

Development of the laser printer as a generalized graphic output device continues. The printer can now handle any standard format graphics file, and it is therefore possible to print ad hoc graphics, such as curves and plots. Specialized graphs, such as chemical structures, can be printed uneventfully and the ability to print these two types of graphics, fulfills most of the DTP needs in this area. The only problem with the laser printer is that demand for such output is high and is close to the capacity of the system. If demand increases further, some expansion of capacity will be necessary.

Signatures are a special type of graphic and work has been completed to store the signatures of various DTP staff so that they can be retrieved and appended to letters as necessary. Each signature is accorded a specially high degree of protection so their unauthorized use is not possible.

2. Use of Robotics in in-vitro testing.

The ITB has acquired a robot and is exploring the possibility of its use in the in-vitro testing environment. The robot can read the label on a sample vial, transfer its entire contents to a labelled, tared vial and weigh the sample. In this way, a file of NSC number versus weight for a sample may be measured. This is done with no human intervention at a rate which allows about 500 samples to be processed in 24 hours. It seems likely that this can be accelerated somewhat, and the fact that the robot can operate in a cooled, sterile, anaerobic, dark environment offers some significant advantages.

Work has begun to examine the use of the robot in the drug addition step of the *in vitro* screening. This is rather more complicated, but it seems clear that the robot can load plates with multiple dilutions of a solution quite efficiently and this may prove to be a valid application.

Publication:

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ANNUAL REPORT OF THE PHARMACOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The newly formed Branch directed its efforts this year mainly in two areas of interest to DTP. Pharmacology and Detailed Therapeutic Evaluations of anti-tumor drugs indicated by various program committees to be candidates for further development. The Master Agreement Task Order contract mechanism for pharmacology was used to initiate or complete studies in three phases for each drug: 1. Analytical Methods Development and In Vitro Stability and Plasma Binding. 2. Pharmacokinetics following single bolus doses. 3. Pharmacokinetics following continuous administration. Task Order Contract studies were completed on the following drugs in 1985/86.

Pibenzimol NSC 322921
Caracemide NSC 253272
5-Azacytidine NSC 102816
Phyllanthoside NSC 328426
Aphidicolin Glycinate NSC 303812
L-Cysteine Derivative NSC 303861
Merbarone NSC 336628
Deoxyspergualin NSC 356894
Teroxirone NSC 296934
Melphalan NSC 8806

Task Order Contracts were initiated and are ongoing on the following drugs in 1985/86.

Anthrapyrazole NSC 349174
Pyrazine Diazohydroxide NSC 361456
Cyclodisone NSC 348948
Clomesome NSC 338947
Chloroquinoxaline Sulphonamide NSC 339004

The Branch exerted a considerable effort in relating therapeutic effects, pharmacokinetics and toxicological information of several drugs in coordinated collaborations with the Toxicology Branch, Pharmaceutical Resources Branch, CTEP personnel and FDA. This effort centered primarily around three drugs (Flavone Acetic Acid, Merbarone, Deoxyspergualin) which had IND filing in 1985/86 but continues with all promising drugs. Two manuscripts on Flavone Acetic Acid have been submitted for publication as a result of this effort.

Detailed Schedule and Therapeutic Evaluation studies were initiated this year using Alzet mini pumps for constant infusions in mice. Experiences with Deoxyspergualin (DSG) indicate that useful information may be obtained using this mode of administration. The best therapeutic response was seen in i.p. implanted L1210 leukemia when s.c. constant infusions of DSG were conducted for 72 hrs, additional results indicated that longer infusions at the same infusion rate were toxic.

In addition to Deoxyspergualin, preclinical therapeutic summaries for Merbarone and Flavone Acetic Acid were prepared for CTEP for inclusion in IND applications and clinical brochures.

Detailed evaluations both in vitro and in vivo were conducted on a variety of tumor types with a number of agents, listed below, to investigate concepts of exposure time, resistance, route of administration, schedule and mechanism of action.

Rhizoxin NSC 332598
Clomesome NSC 338947
Chloroquinoxaline Sulphonamide NSC 339004
Cyclodisone NSC 348948
Pyrazine Diazohydroxide NSC 361456
Tetraplatin NSC 363812

In addition to the above studies some testing of congener series of a variety of drugs in cooperative efforts with the Drug Synthesis and Chemistry Branch and Natural Products Branch was carried out this year. Special non-standard protocol studies were conducted to answer questions which arise during analogue development or extraction processes.

Publications by Staff

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5. Zaharko, D. S., Covey, J. M. and Muneses, C. C.: Experimental Chemotherapy (L1210) with 5-aza-2'-deoxycytidine in combination with pyran copolymer (MVE-4), an immune adjuvant. JNCI 74: 1319-1324, 1985.

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Publications by Contractors

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ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The mission of the Developmental Therapeutics Program centers on the discovery and preclinical development of agents with clinical anticancer potential. Investigations focusing on the hazards of antineoplastic compounds to healthy organs in intact experimental animals are the final steps in the preclinical stages of new drug development. Such investigations comprise the primary responsibility of the Toxicology Branch. Toxicology studies designed to meet this responsibility involve three major objectives:

1. Determination and safety assessment of an initial dose for clinical trial.

2. Determination of primary organ systems adversely affected by drug administration.

3. Determination of the reversibility of the adverse effects.

Data generated from studies on each new drug and evaluated in light of potential human toxicity comprise the major portion of the evaluable information required by the Food and Drug Administration for an Investigational New Drug Application.

During this fiscal year the direction of the Toxicology Branch has continued on two highly integrated paths. The first path is the elucidation of the potential adverse effects of new anticancer agents. The second path is the acquisition and use of pharmacokinetic information to reliably extrapolate toxic effects across species by relating plasma drug levels (peak and steady state) to the appearance and severity of toxicity as well as to assess the schedule dependency of toxicity. Integration of these two courses permits a more rational evaluation of the role of schedule dependence in efficacy of new agents as well as in development of toxicity.

In the main, the Branch achieves its primary responsibility through the operation and management of a prime contract in which the qualitative and quantitative toxicological profiles of antitumor drugs and modalities are determined in experimental animals. For management, the prime contract is divided into four definitive tasks. Task I is devoted to the complete preclinical toxicologic evaluation of cytotoxic agents, radiosensitizers, radioprotectors, etc. Standardized guidelines using mice, rats and dogs are followed to determine the initial dose for Phase I clinical trials, to verify safety of the initial clinical dose and to elucidate specific target organ toxicity and its reversibility. Task II studies are concerned with limited evaluations of drugs. These studies are performed to complete the toxicity profile on compounds for which some toxicology data is available. Task III involves the development and implementation of *in vivo* and *in vitro* tests to evaluate organ specific toxicity. The "special studies" carried out under this task yield important information

leading to development of new, more meaningful toxicity testing studies. Task IV of the Prime Contract deals with the administrative aspects of toxicity testing such as data handling, subcontractor monitoring as required by Good Laboratory Practice Regulations, and financial and program management.

The Division of Cancer Treatment maintains a master file with the FDA which contains toxicity testing protocols for antitumor agents. These protocols set forth jointly agreed to procedures for animal toxicity testing of antineoplastic drugs. Data from studies conducted under the protocols are accepted for regulatory purposes in INDA approval. The Toxicology Branch has amended the protocols to accurately reflect newer methods and techniques. Additionally, individualized protocols (drug specific) are routinely developed to account for agent specific chemical and/or physical properties. During the past year, the Branch has collected and evaluated pharmacokinetic data in addition to toxicity data in an effort to understand the in vivo behavior of new agents related to toxic findings. New guidelines, dependent on drug characteristics, for pharmacokinetic studies and continuous delivery procedures in mice and dogs have been submitted to the Food and Drug Administration for inclusion in the Master File.

Data on the following agents have been, or are anticipated to be, presented to the Decision Network Committee in FY'86:

Oxanthrazole	NSC-349174
Fazarabine	NSC-281272
Chlorsulfaquinoxaline	NSC-339004
4-Ipomeanol	NSC-349438

Toxicology studies have been, or are anticipated to be, initiated in FY'86 on the following new antineoplastic agents:

L-Buthionine Sulfoximine	NSC-326231
Phyllanthoside	NSC-328
Clomesone	NSC-338947
Discreet Agent	NSC-320846D

Studies on these agents will be completed during FY'87 and will provide a complete picture on their toxic effects and pharmacokinetic behavior using the integrated approach developed over the past two years.

The Developmental Therapeutics Program has taken the lead role in the development of new antiAIDS drugs. The Toxicology Branch is thus intimately involved in the development of toxicology studies for the new agents and the performance of the studies for filing INDA's with the Food and Drug Administration. To date, two drugs have been scheduled for toxicity testing: Dideoxycytidine and Dideoxyadenosine. The data on the continuous infusion studies of Dideoxycytidine are scheduled to be submitted to the Food and Drug Administration in July, 1986.

In order to facilitate the toxicology characterization of these new compounds, a separate contract laboratory network has been established to test new agents active against the AIDS virus.

Complete protocol guidelines have been developed specifically for each agent. The guidelines include, but are not limited to, the three phases as outlined below:

1) Analytical Phase - Drug identity analysis, validation of the procedures supplied by the NCI for dose concentration analyses and validation of the requisite methodology for assay of drug in biological fluids will be initiated immediately upon receipt of drug.

2) Acute Toxicity and Pharmacokinetic Phase - During this phase, ip or sc or po bioavailability will be determined in a rodent species and the dog establishing the dose required to produce the virus inhibitory concentration (VIC) in plasma. Plasma elimination pharmacokinetics in a rodent species and dogs will also be carried out as will limited acute (single dose) toxicity studies.

3) Toxicity Phase - During this phase organ toxicity of the drug will be determined. Drug will be administered for 2 and 4 weeks with a 6-8 week recovery period. The routes of drug administration may be intravenous, intraperitoneal or oral. In general up to three dose levels per species (fractions or multiples of the VIC dose) will be used.

It is anticipated that four to five new agents will be evaluated each year, with similar numbers of clinical trials established for each agent as in the past.

Publications and Presentations

Staff

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Contractors

During this year contractors and subcontractors to the Toxicology Branch presented 6 papers which involved NCI support.

ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Pharmaceutical Resources Branch (PRB) is structured to provide comprehensive pharmaceutical services to the various Programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. During this report period the Branch supervised a total of 20 contracts with a combined annual budget of approximately 6.7 million dollars.

The major contract areas include: chemical preparation and pilot plant production, analytical services, pharmaceutical research and development, and pharmaceutical manufacturing. Additionally, the Branch is responsible for shelf life surveillance, storage and distribution, and computerized inventory maintenance of all drug products used in the Clinical Programs of the Division of Cancer Treatment (DCT).

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high Program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal Chemistry are assigned to the formulation laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Staff

The Pharmaceutical Resources Branch is presently staffed with six senior professionals, one technical and two secretarial personnel. In addition, one visiting fellow is assigned to the formulation laboratory. The classification of the senior professionals is as follows: four PHS Commissioned Corps Pharmacists, one Ph.D. analytical chemist, and one Ph.D. medicinal chemist.

The Branch consists of three Sections:

1. Chemical Resources Section

The primary functions of the Chemical Resources Section are to provide for resynthesis, large-scale production and procurement services for the acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for resynthesis and pilot plant production of various quantities of bulk substances intended for tumor screening panels, preclinical toxicology and pharmaceutical manufacture of investigational products for clinical evaluation.

The chemical preparation laboratory provides the means of obtaining a broad variety of chemical compounds, and the ability of providing large quantities

of high purity drug substances. The Section supervises seven chemical prep lab contracts consisting of a combined annual effort of approximately 34 man years.

2. Analytical and Product Development Section

This Section has two major functions: (1) the analytical assessment of chemicals and formulated products, and (2) the development of investigational pharmaceutical dosage forms for clinical trial.

The analytical component of the Section supervises contractors engaged in the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions, and identification of impurities and/or degradation products. The Section's staff prepares bulk chemical specifications used for acceptance criteria of additional supplies either from commercial sources or chemical preparation contractors. The specifications and validated analytical methodology are prepared in a format suitable for submission to the Food and Drug Administration as part of the NCI's Investigational New Drug Application. The Section is also responsible for chemical evaluation of new investigational dosage forms.

Presently, the Section's analytical chemist supervises three analytical contracts representing a combined 18 staff year effort. These contractors have the expertise to chemically characterize a very structurally diverse group of chemicals. These contractors are also responsible for the development and application of stability-indicating methods for all new drug substances.

The dosage form development component of the Section is responsible for conversion of bulk chemical into a pharmaceutical product suitable for clinical use. Essentially all products are developed for intravenous use. About one-half of the drugs do not exhibit adequate solubility or stability and some form of pharmaceutical intervention is required. Standard approaches (salts, solvents and surfactants) are initially evaluated. Emphasis is also given to evaluation of newer techniques to improve solubility or stability (emulsions, prodrugs and complexation). The developed dosage form is evaluated for chemical content, antitumor activity in the rodent screen, and feasibility to be manufactured on production scale.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory, supervised and maintained by this Section, is investigating methods to solve drug formulation problems.

The Analytical and Product Development Section is responsible for the supervision and management of eight contracts: three analytical contracts with a combined annual effort of 18 man years, three pharmaceutical R & D contracts with a combined annual effort of eight man years, one combined R & D (one and one-half man years), and a pharmaceutical contract.

Analytical data developed on new investigational compounds is assembled and published in a book entitled "NCI Investigational Drugs - Chemical Information." This text contains stability-indicating methods, spectral data, approximate solubility and stability data, and other appropriate information on a large number of agents. The publication is distributed to investigators throughout the country.

3. Clinical Products Section

The Clinical Products Section manages four pharmaceutical contracts with capabilities to produce a broad variety of pharmaceutical products. The Section manages a storage and distribution contract with computer capabilities for accurate accountability of the disposition of all investigational products, and also manages a shelf life contract involving an annual three and one-half man year effort.

In addition, the Section manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this report period, drug purchase expenditures were in excess of two million dollars. A similar effort was begun during the year to record the expenditures for drugs for patients with acquired immune deficiency syndrome (AIDS). This involved establishing a dual record system to accurately account for the expenditures for AIDS drugs.

A significant amount of staff time is expended in this pursuit in preparing purchase specifications, award justifications, and performing financial record-keeping functions. Several different NIH mechanisms to obtain contracts are utilized to obtain drugs, such as, blanket purchase agreements, indefinite delivery contracts, direct purchase contracts, etc.

Investigational product literature in the form of Investigational Drug - Pharmaceutical Data Sheets is prepared by the Section. These information sheets are also supplied in bound book form (NIH Publication No. 86-2141) which is updated periodically. During this reporting period, over 4,300 issues were distributed.

The contractors managed by the Clinical Products Section produced over 425,000 injectable units, and slightly less than 200,000 oral dosage forms for clinical distribution.

Goals and Accomplishments

The Pharmaceutical Resources Branch has successfully accomplished its objectives in providing high quality bulk chemicals and pharmaceutical products to the various Programs in the Division of Cancer Treatment. During this reporting period, the prep lab contractors prepared over 100 compounds totaling more than 250 kilograms. Examples of bulk pharmaceutical substances delivered included: L-histidinol (NSC-74518), HMBA (NSC-95580), Dideoxycytidine (NSC-606170), Merbarone (NSC-336628), and Buthionine-S, R-sulfoximine (BSO) (NSC-326231).

The analytical contractors have submitted reports describing the analysis of over 100 lots of bulk chemicals and formulated products. Methods were developed for the detection of the following drugs in mouse and human plasma, and growth media: BSO (NSC-326231), Chloroquinoxaline sulphonamide (NSC-339004), and Ebifuramin (NSC-201047).

Pharmaceutical research and development activities were also quite successful in completing about twelve dosage form development projects. Among the completed projects were new formulations of Clomesone (NSC-338947), BSO (NSC-326231), Perilla Ketone (NSC-348407), and Triazine Antifol (NSC-127755) and (NSC-D338720). Compounds exhibiting significant solubility and stability problems continue to be encountered. A multistage approach is used to resolve these problems. Emphasis has been given to parenteral oil/water emulsions as a non-specific means of improving solubility.

The Biological Response Modifier Program continues to increase in the use of new agents to treat cancer. Several new agents entered clinical trial during the reporting period, i.e. Leuprolide, Human Tumor Necrosis Factor, Photofrin, Zolodex, and several new monoclonal antibodies. The magnitude of this program continues to grow and requires much staff time to maintain adequate inventories of these various agents.

Several new agents for the treatment of patients with AIDS entered clinical trial, i.e. Suramin and Azidothymidine. Clinical supplies of Azidothymidine were limited and required close monitoring by the Section to ensure that a continuous supply was made available to the NCI clinicians.

In response to OSHA's regulations and new state laws governing the worker's "right to know" laws, dealing with the safe handling of toxic substances, the Branch began preparing material safety data sheets on the drugs provided to our contractors. This effort will be in collaboration with the Division of Safety, Toxicology Branch, and other Divisions within the Institute.

The Section was requested to derive a cost accounting system to capture all the costs that go into the production of a product. A system was developed that would allow a cost to be assigned to each and every vial or bottle of capsules provided by the Branch. These costs are utilized by the Cancer Therapy Evaluation Program as part of the protocol review process.

The number of companies providing drugs at no expense to the government increased over the past year. The NCI received approximately 315,000 vials/ampules and over 160,000 tablets/capsules for use in NCI/sponsored trials. Some examples are as follows: Roche, Burroughs Wellcome, Schering, and Genentech: various interferons; Roche and Cetus: Interleukin; Adria: Adriamycin and Esorubicin; Upjohn: Menogaril; Burroughs Wellcome: Leucovorin and Azidothymidine; Degussa: Mesna, etc.

Publications by Staff

1. Cradock, J.C., Vishnuvajjala, B.R., Chin, T.F., Hochstein, H.D., and Ackerman, S.K.: Uridine-induced hyperthermia in the rabbit. J. Pharm. Pharmacol. 38: 226-229, 1986.

2. Vishnuvajjala, B.R. and Cradock, J.C.: Tricyclo [4.2.2.$0^{2,5}$] Dec-9-ene-3,4,7,8-tetracarboxylic acid diimide (NSC-284356) Formulation and Stability Studies. J. Pharm. Sci. 75: 301-303, 1986.

3. Trissel, L.A., Davignon, J.P., Kleinman, L.M., Cradock, J.C., Flora, K.P., and Quinn, F.R.: NCI Investigational Drugs - Pharmaceutical Data 1986. NIH Publ. No. 86-2141, 181 pp., 1986.

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6. Foster, B.J., Clagett-Carr, K., Shoemaker, D.D., Suffness, M., Plowman, J., Trissel, L.A., et al: Echinomycin: The First Bifunctional Intercalating Agent in Clinical Trials. Invest. New Drugs 3: 403-410, 1986.

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8. Chun, H.G., Leyland-Jones, B., Hoth, D., Shoemaker, D.D., Wolpert-DeFilippes, Grieshaber, C., Cradock, J.C., Davignon, J.P., Moon, R., Rifkind, R., and Wittes, R.: Hexamethylene bisacetamide: A polor-planar compound entering clinical trials as a differentiating agent. Cancer Treat. Rep. 1986, in press.

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11. Davignon, J.P., and Cradock, J.C.: The Formulation of Anticancer Drugs. In K. Hellman and S.K. Carter (Eds.) Fundamentals of Cancer Chemotherapy, McGraw-Hill, 1986, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-03584-14 PRB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Research in the Development of Dosage Forms of New Antitumor Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	James C. Cradock	Head	A&PDS	PRB	NCI
	Karl P. Flora	Chemist	A&PDS	PRB	NCI
	Aaron Garzon	Visiting Fellow	A&PDS	PRB	NCI

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Pharmaceutical Resources Branch

SECTION

Analytical and Product Development Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project describes the activities of the formulation laboratory of the Pharmaceutical Resources Branch. These studies are directed toward resolving problems in the intravenous delivery of antitumor agents and involve methods to evaluate and then improve drug solubility and stability.

A series of water soluble prodrugs of camptothecin have been prepared. These compounds were evaluated with respect to solubility behavior, chemical stability, and rate of hydrolysis in mouse and human plasma. In vivo antitumor activity was also assessed in comparison to camptothecin. The solubilities of the prodrugs were about 1000 fold higher than the parent compound. All of the compounds are reasonably stable in aqueous media (t 1/2 > 10 hours at room temperature). In the presence of plasma, the compounds readily hydrolyze (t 1/2 = 10-90 minutes at 37°C). The rates in human plasma were about twice as fast as in mouse plasma. Equivalent antitumor activity was noted at dose levels about two to four fold higher than camptothecin.

The pharmaceutical behavior of the hexamethylmelamine analogue, trimethylmelamine was evaluated. The compound can be formulated on an extemporaneous basis in aqueous solution but decomposes so rapidly that manufacture on large scale is not practical. Several methods of drying from water miscible organic solvents were investigated. Since the compound is more stable in ethanol than in water, low temperature vacuum drying from ethanol was found to yield a stable dry product.

PHS 6040 (Rev. 1/84) GPO 814-818

Camptothecin is a poorly water soluble natural product that is very active in several experimental tumor models. The sodium salt of the lactone is active but at tenfold higher doses. Wani et al. noted that an intact lactone group was required for optimal activity (J. Med. Chem. 23: 554-560, 1980). The purpose of this study was to prepare water soluble prodrugs ($\geq$ 5 mg/ml) that would revert to the parent compound chemically or enzymatically and exhibit comparable activity to camptothecin at equivalent doses.

All prodrugs involved substitution at the hydroxyl group of position 20. A series of monosubstituted glycine esters (methyl, ethyl, propyl, and butyl) as HCl salts were prepared and compared to the glycine HCl ester. The water solubility of these compounds was in the range of 5-7.5 mg/ml and was adequate to deliver the antitumor doses at 1-2% body weight.

Since the solubility of the parent compound is at least two orders of magnitude less than the prodrugs, a slight degree of conversion back to the parent compound could exceed its aqueous solubility and result in formation of a precipitate. These compounds reverted back to camptothecin at room temperature, but the rate was sufficiently slow (t 1/2 > 48 hours) to avoid precipitation during the anticipated pharmaceutical processing time. A pH versus stability profile indicated the reaction rate was faster in acidic media. These compounds appear to exhibit the pharmaceutical properties required to overcome the solubility problems. We had previously demonstrated that the magnitude of the solubility requirement for camptothecin was beyond the usual formulation approaches: solvents, surfactants, complexes, and emulsions.

The effect of incubation with plasma on decomposition of these compounds was also evaluated. The half-life in mouse plasma ranged from about 30 minutes (glycine and mono-methyl derivative) to 90 minutes (mono-butyl substituent). In human plasma the reaction rates were approximately twice as fast. The data indicate that the conversion is enzymatic and that these compounds are behaving as prodrugs. However, these rates are determined by measuring disappearance of the derivatives over time. Camptothecin was detected, the recovery was not quantitative presumably due to the strong protein binding of this drug. The activity versus i.p. L_{1210} was similar to camptothecin, but all compounds were two to four fold less potent. Although the substituted glycine derivatives were easier to prepare, no other advantages were evident over the glycine ester.

Subsequently, lysine, glutamate, hemisuccinate, and phosphate esters were prepared and similarly evaluated. These derivatives hydrolyzed more readily in water (t 1/2 14-38 hours). Lysine and hemisuccinate esters exhibited higher water solubility (13-15 mg/ml). No substantial differences in reaction rates were noted in the plasma incubation experiments, and evaluation of antitumor activity is currently ongoing.

A number of water soluble camptothecin derivatives have been prepared with an intact lactone function. These compounds appear to behave as prodrugs and exhibit satisfactory pharmaceutical properties. Currently, some of these prodrugs are being tested intravenously versus the L_{1210} to better estimate their potential utility.

Trimethylmelamine is a water soluble analogue of hexamethylmelamine that is under clinical evaluation by the EORTC, and a potential candidate for studies in the Unites States. This compound is very unstable in water. Consequently, clinical batch sizes have been very small. An alternative approach was required to increase stability and permit manufacture on a production scale. The solubility and stability of trimethylmelamine is greater in ethanol than in water, and a stable dry dosage form was prepared by evaporation of a trimethylmelamine solution in ethanol using the low temperature vacuum drying technique in a conventional freeze dryer. At time of use the sterile dry drug could be constituted with sterile DMSO, diluted to 10 mg/ml (5% DMSO) with 5% dextrose in water and administered by infusion over 60-90 minutes.

Several small projects were also carried out to assess the magnitude of the formulation problem presented by selected compounds. One example involves NSC-378965. In view of previous work with mitindomide, the solution behavior of this water soluble prodrug is being evaluated using a recently developed HPLC method.

Publications

1. Cradock, J.C, Vishnuvajjala, B.R., Chin, T.F., Hochstein, H.D., and Ackerman, S.K.: Uridine-induced Hyperthermia in the Rabbit. J. Pharm. Pharmacol. 38: 226-229, 1986.

2. Vishnuvajjala, B.R. and Cradock, J.C.: Tricyclo(4.2.2.$0^{2,5}$) dec-9ene-3,4, 7,8-tetracarboxylic acid Diimide: Formulation and Stability Studies. J. Pharm. Sci. 75: 301-303, 1986.

Annual Report of the Office of Extramural Resource Analysis and Development

Developmental Therapeutics Program

Division of Cancer Treatment

October 1, 1985 to September 30, 1986

This newly formed group has been established to provide an administrative and managerial focal point for all DTP extramural activities. These activities encompass a) contracts b) grants and c) cooperative agreements such as those involving the National Cooperative Drug Discovery Groups (NCDDG). The activities of the office during the year are summarized below in the funding categories.

Contracts

Forty RFPs were issued and/or awarded during the year and these are listed in Table I together with title of the solicitation and associated dollars.

Grants

The Biochemistry-Pharmacology Program grants portfolio is shown in Table II for this year.

Cooperative Agreements

NCDDG - The first two anticancer NCDDG awards were made in 1984 and are currently in their third of five planned funding years. Two additional anticancer NCDDG awards were made in 1985. The recipients of these awards, anticipated FY 1986 total funding, and Group objectives are listed below.

1. Roswell Park Memorial Institute (1 U01 CA-37606-03), $615,543 - "Antitumor Treatment Potential of Inhibitors of Polyamine Biosynthesis and Function"

2. Northern California Cancer Program (1 U01 CA 37655-03), $383,185 - "Approaches to the Inhibition of Oncogene Expression"

3. University of Florida (1 U01 CA 49884-02), $616,543) - "Topoisomerases as New Anticancer Therapeutic Targets"

4. Memorial Sloan Kettering Cancer Center (1 U01 CA 376431-02), $608,113) - "Anti-receptor Monoclonal Antibodies in Cancer Treatment"

Thus, the four current anticancer NCDDGs involve 21 laboratory programs carried out in 14 institutions with planned FY 1986 funding at $2,223,384.

During the current year, OERAD issued an RFA for NCDDG for Treatment of AIDS. NCI and NIAID have jointly set aside $3,000,000 for the FY 1986 awards. Twenty applications composed of over 70 laboratory programs were received. The review process is expected to be completed in June 1986.

TABLE 1

Total Contract Value (Estimated/Awarded)

Antitumor Projects

Provision of Animal Facilities and Performance of Routine Tests	$ 1,359,258
Synthesis of Congeners and Prodrugs	2,545,042
Preparation of Radiolabeled Materials	973,813
Surveillence of Selected and Promising Natural Products	359,051
Large Scale Isolation of Antitumor Agents from Natural Sources	630,500
Animal Diagnostic Laboratories	1,780,649
Evaluation of Congeners of New Lead Compounds	934,163
Support Services in Virology, Tissue Culture and Immunology	1,227,364
Deep Sea Marine Organism Collection	1,892,132
Shallow Water Marine Organism Collection	1,657,690
Cultivation of Blue-green Algae	1,382,975
Plant Collection Program	1,287,470
Storage and Distribution of Clinical Drugs	2,054,146
Development and Production of Pharmaceutical Dosage Forms	1,125,214
Development of Dosage Forms and Delivery Systems for New Antitumor Agents	1,418,625
Primary Rodent Production Centers	14,753,700
Computer Support Task	999,611
Preparation of Bulk Chemicals and Drugs for Phase II and III Clinical Trials	7,538,446
Quality Control of Rodents and Tumor Cell Lines	1,500,892
Master Agreement for Chemical Synthesis	1,350,000
Preclinical Pharmacology Investigations of Antitumor Agents	1,917,667
Synthesis of Compounds for Preclinical Toxicology and Phase I Clinical Studies	3,650,625
Services in Support of the Developmental Therapeutics Program	519,139
Computer Based Searches for Chemical Structures	461,391
Detailed Drug Evaluation and Development of Treatment Strategies for Chemotherapeutic Agents	1,733,875
Production of Clinical Doses of Antitumor Agents	4,973,067
Analysis of Chemical and Pharmaceutical Formulations	5,948,336
Storage and Distribution of Chemicals & Drugs Used in Prelinical Evaluation and Development	2,718,612
Provisions of Hematopoietic Cell Cultures, Growth Factors and Type C Virus Protein	3,207,600
Preparation and Supply of Fresh and Cultured Mammalian Cells	730,803
Provision of Tissues and Cells and Conduct of Routine Tests in Support of Tumor Cell Biology Studies	3,676,896
Preparation & Purification of Viral Components	1,014,053
Operation of an Animal Virological Diagnostic Laboratory	1,381,407
Subtotal	78,704,212

Anti-AIDS Projects

Development and Manufacture of Oral Dosage Forms of Anti-AIDS Agents	3,152,500
Dosage Form Development of New Agents for the Treatment of AIDS	472,875
Analysis of Chemicals and Pharmaceutical Formulations for Anti-AIDS Agents	1,933,972
Development and Production of Parenteral Dosage Forms of Anti-AIDS Agents	6,305,000
Large Scale Preparation of Anti-AIDS Drugs for Preclinical Toxicology & Phase I Clinical Studies	2,364,375
Large Scale Preparation of Anti-AIDS Bulk Drugs for Phase II and III Clinical Trials	4,728,750
Preclinical Toxicology Studies of Anti-AIDS Agents	5,033,859
Subtotal	23,991,331
Grand Total	$102,695,543

TABLE 2

OERAD GRANTS PROGRAM
(PROVIDED BY EFDB)

Biochemistry and Pharmacology Program
by Sub-Category
FY 1986 (Estimated)

	Number of Grants*	Total Amount (Thousands)
Synthesis and Chemistry	71	8,815
Natural Products	49	6,173
Screening and Experimental Therapeutics	36	4,178
Comparative Pharmacology	21	2,210
Other Preclinical Aspects	6	725
Mechanism of Action	100	13,192
Program Projects	8	8,541
Total	291	43,834

*Includes: Traditional (RO1)
New Investigator (R23), Small Business Innovative Research (R43/R44), Academic Research Enhancement (R15), Awards and supplements to the foregoing. Does not include Conference (R13) Awards.

ANNUAL REPORT OF THE LABORATORY OF BIOLOGICAL CHEMISTRY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Laboratory of Biological Chemistry was established in 1985 to identify as targets for drug design, cellular reactions that are critical to the control of tumor cell proliferation or differentiation. Recent advances in cell biology are evaluated for possible targets. Agents are designed to interact with these targets and are evaluated for biochemical and antitumor effectiveness. An important aspect of this mission is to develop appropriate *in vivo* systems to evaluate the chemotherapeutic effectiveness of agents shown to be active in simpler *in vitro* model systems. Accordingly, the Laboratory is involved in identifying endogenous factors present *in vivo* that modify drug action and influence differential toxicity with the aim of manipulating these factors to enhance antitumor activity. Approximately half of the Laboratory's resources is applied to non-traditional targets for antitumor drug design and study. These non-traditional targets include early key biochemical events signaling cell proliferation or differentiation. The other half of Laboratory resources is applied to the study of either traditional targets or active compounds with traditional or unknown mechanisms of action.

Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated projects to develop new chemotherapeutic agents to block the action of growth factors. Non-traditional targets selected for drug design and study include: second messengers inositol triphosphate and diacylglycerol; myristoylation of cellular oncogene products; protein kinase C; and the *ras* oncogene product.

Recent findings indicate that inositol-phosphates formed from phosphatidylinositides may be the second-messengers which mediate the action of many growth factors. In addition, the product of two distinct oncogenes (src and ros) phosphorylate phosphatidylinositol and increase the levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. The synthesis of phosphatidylinositol polyphosphates and their subsequent hydrolysis therefore provide attractive well-defined targets for the design of inhibitors for use in chemotherapy. A project was initiated that has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell replication. Secondly, to design, synthesize, and evaluate drugs to inhibit this process, specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme which produces the inositol phosphates. Test systems have been developed to

identify drugs that inhibit phosphatidylinositol turnover or synthesis. During this period we have completed development of a new method for the separation and measurement of inositol phosphates. This technique has revealed that the metabolism of these compounds is more complex than previously known, and involves multiple positional isomers. Current studies are exploring the role of these compounds in growth factor action. Uptake studies revealed that myo-inositol enters L1210 cells by a process not saturable to at least 5 mM, and reaches an intracellular concentration approximately equal to the external concentration. Our initial efforts in drug development have centered on a series of myo-inositol analogs. We have developed a structure-activity profile for the incorporation of these analogs into fraudulent PI analogs.

It may be possible to alter the activity of an oncogene product by interfering with its localization in the plasma membrane. The early events signaling cell proliferation occur in the plasma membrane of the cell, the location of most of the known cellular oncogene products. Myristoylation has been shown to be critical for the membrane localization and cellular transforming activity of p60src and has been implicated for other transforming proteins. Three mechanisms for blocking myristoylation-dependent transformation are under investigation: (1) direct chemical inhibition of transforming protein myristoylation; (2) inhibition of translocation of the myristoylated transforming protein to the plasma membrane; and (3) displacement of the myristoylated transforming protein away from the membrane, into the cytoplasm, and thus away from its substrate. Methods developed in the p60src system will be applied to malignant cells where membrane-associated oncogene products are suspected.

Inducers of protein kinase C activity such as diacylglycerols and phorbol esters, have shown the importance of this enzyme in the regulation of growth factor activity, lymphoblastogenesis, normal cell proliferation, cellular differentiation and oncogene expression. Calcium- and phospholipid-dependent protein kinase (PK-C) activity was determined in association with the differentiation response of HL-60 leukemia cells to DMSO, retinoic acid, 1,25-dihydroxyvitamin D_3, phorbol ester (TPA) and interferon-γ. PK-C activity rose in proportion to the appearance of the mature granulocytic or monocytic phenotype. Conditions under which TPA produced the macrophage phenotype resulted in disappearance of PK-C, but duplication of this phenotypic response with one hour priming doses of TPA and post treatment with retinoic acid showed conclusively that down regulation of PK-C is not a necessary consequence of the macrophage phenotype. A new investigation has been initiated to study the regulation of plasma membrane-associated tyrosine kinase in HL-60 leukemia cells undergoing differentiation and in cells resistant to differentiating agents. In addition, the relationship between membrane tyrosine kinase and pleiotropic drug resistance in human breast carcinoma cell line MCF-7 is being investigated to assess the association between the p170 glycoprotein induced in multidrug resistant cells, calcium channels and tyrosine kinase. To investigate these problems, a new nondenaturing gel electrophoretic assay for multiple tyrosine kinase activities in cell extracts has been developed.

A new study was initiated to determine the function of the ras oncogene product as a target for drug design. Activation of the ras oncogene has been implicated as the causative agent in as many as 30% of all human tumors. Yet in spite of extensive work on the ras gene, including the identification of at least seven different ras genes and an increasing number of ras-like genes,

almost nothing is known about the function of the ras proteins in higher eukaryotes. A recently characterized protein, termed ARF, is a component of the adenylate cyclase system and shares several features with ras, including size, location, and ability to bind GTP. Characterization of the binding and hydrolysis of guanine nucleotides by ras and ARF has begun. ARF was purified from bovine brain membranes. Cyanogen bromide fragments of the pure protein were separated and two amino acid sequences of about 25 residues each were obtained. Synthetic peptides were synthesized for injection into rabbits to produce specific antibodies to ARF. Oligonucleotide probes were also synthesized from the amino acid sequence and are being used to screen a cDNA library to obtain the full length sequence of ARF. These antibody and cDNA probes should help to identify the site and physiological role of this novel membrane protein. Comparison of the primary and predicted tertiary structure of ARF to other recently cloned G-proteins may aid in the identification of specific domains involved in binding other proteins or binding and hydrolyzing GTP. In this case, site-directed mutagenesis will be used to construct specific altered proteins to test for cellular functions of ARF. A systematic search will then be conducted to identify factor(s) which increase either the exchange or hydrolysis of guanine nucleotides by these regulatory proteins. These studies should locate cellular targets for these proteins and may identify the physiological role of these proteins in cellular metabolism or proliferation.

The availability of tissue culture cell lines has made it possible to study the regulation of proliferation and differentiation of specific hematopoietic cell types and the effects on these cells of known or suspected mediators and modulators. HL60 has been a useful model system in the search for substances that are active as inducers of differentiation. HL60 is induced to differentiate to granulocyte-like cells by incubation with retinoic acid (RA), DMF, and DMSO or into monocyte/macrophage-like cells by incubation with 1,25-dihydroxyvitamin D_3 and TPA. Of the many compounds that induce differentiation of HL60, RA has probably the most promise of being of use in the clinic. To the extent that it is possible, results in vitro should suggest treatments in vivo. To this end we have studied the differentiation effect of RA in nude mice carrying a transplantable HL60 tumor. There was no increase in life-span of treated animals and no evidence that tumor cells were induced to differentiate. These results were even more surprising because the tumor cells, established in culture and designated HL60/MRI, were found to be 100-fold more sensitive to RA in vitro than the parent HL60. In addition, RA induces HL60/MRI to differentiate to monocytoid cells. RA binds to serum albumin in a specific manner with an equilibrium constant of association of $10^6 M^{-1}$. Thus, even at pharmacological concentrations of 1 μM all of the circulating RA is bound to albumin. This binding may be a major factor for the absence of antitumor activity of RA in vivo as serum and/or serum albumin inhibit RA induced differentiation of HL60 in vitro.

Multidrug-resistance is a well documented phenomenon that limits the chemotherapeutic effectiveness of many traditional antitumor agents. An understanding of the physiologic function of proteins associated with multidrug-resistance could lead to the design of more effective chemotherapeutic strategies with existing agents or the associated proteins may be considered as targets for drug design, in which case a new class of antitumor agents might arise. During the past year an important advance was made in our Laboratory

in the form of a tool to study the biology of the multidrug-resistant protein(s) and which ascribes a functional role to these proteins. Photoactive radioactive analogues of vinblastine were used to photo-affinity label membranes of Chinese hamster lung drug-sensitive (DC-3F), multidrug-resistant sublines selected for resistance to vincristine (DC-3F/VCRd-5L) or actinomycin D (DC-3F/ADX), and revertant (DC-3F/ADX-U) cells. A radiolabeled doublet (150-180 kDa) consisting of a major and minor band which was barely detectable in parental drug-sensitive cells was increased up to 150-fold in the drug-resistant variants but only 15-fold in the revertant cells. Photoaffinity labeling in the presence of 200-fold excess vinblastine reduced radiolabeling of the 150-180 kDa species up to 96% confirming its Vinca alkaloid binding specificity. The radiolabeled doublet comigrated with a Coomassie blue stained polypeptide doublet in the drug-resistant cells and was immunoprecipitated with polyclonal antibody which is specific for the 150-180 kDa surface membrane glycoprotein (gp150-180) in multidrug-resistant cell lines. The identification of this Vinca alkaloid acceptor in multidrug-resistant plasma cell membranes suggests the possibility of a direct functional role for gp150-180 in the development of multi-drug resistance. These photoactive analogues will serve as useful tools for the isolation of the gp150-180 proteins.

A new agent was synthesized that may have value against slowly growing tumors. The homocysteine derivative, S-methylthio-DL-homocysteine (SMETH) was synthesized and found to be cytotoxic to L1210 cells in culture. Its cytotoxicity was increased in the presence of leucine, which interferes with glutamine utilization as an energy source. A working hypothesis involves the interaction of homocysteine, formed by intracellular disulfide reduction of SMETH, with adenosine, formed by intracellular dephosphorylation of ATP. This mechanism may be applicable for development of chemotherapy of slowly growing or latent tumors.

The methods developed to study the non-traditional targets described above are also being used to study the biochemical effects of new or established antitumor agents. Investigators in this Laboratory have extensive experience in nucleotide metabolism. The mechanism of action of nucleoside analogs was investigated in human carcinoma cell line HT-29, human promyelocytic leukemia cell line HL-60 and L1210 cells. Cyclopentenyl adenosine (neplanocin A) was an effective inhibitor of RNA and DNA methylation in HL-60 cells via its metabolism to an S-adenosylmethionine (AdoMet)-like metabolite. However, neplanocin proved to be a poor inducer of differentiation in HL-60 cells as assessed by nitroblue tetrazolium reduction and cellular myc RNA expression. The 3-deaza analog of neplanocin, c^3Nep, was as effective an inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase as neplanocin but possessed 1/10 the cytotoxicity, did not effectively inhibit RNA methylation and was not metabolized to an AdoMet-like metabolite. c^3Nep was ineffective as a differentiating agent in HL-60 cells. The cyclopentenyl cytidine analog (cCyd) possessed a potent inhibitory effect on CTP synthesis and subsequently, DNA synthesis with moderate cytotoxicity against HT-29 and HL-60 cells. However, this agent proved to be a very effective differentiating agent for HL-60 cells, probably as a result of its S phase specific effects at noncytotoxic concentrations. cCyd was found to have marked in vivo antitumor activity in several murine models. It was especially active against an Ara-C resistant L1210 tumor. Inhibition of CTP synthesis was confirmed in mice bearing L1210 ascites. Cyclopentenyl-ara C and cyclopentenyl-8-aza-adenine were found to be cytotoxic to cultured L1210 cells.

A project involving traditional targets for drug design and study is a continuing project to determine the relative dependency of host and tumorous tissues on de novo vs salvage pathways for the synthesis of pyrimidine and purine nucleotides in vivo. This project is divided into the following specific aims: to determine the physiologic importance of circulating pyrimidines and purines and their role in modulating the antitumor activity of antipyrimidine and antipurine chemotherapeutic agents; to study the liver as a modulator of circulating nucleosides and as a possible target for chemical manipulation; to develop agents to interfere with nucleoside salvage to be used in combination with inhibitors of de novo synthesis; to develop methodology for monitoring and quantitating the flux through the de novo pathways of host and tumorous tissues in vivo.

Studies from our Laboratory demonstrated that low levels of pyrimidines and purines, within the physiological range, are capable of reversing the toxicity of inhibitors of purine and pyrimidine de novo synthesis. Also, at these low concentrations, cultured cells turn off their de novo pathways and use exclusively their salvage mechanisms. Thus, circulating nucleosides may be an important factor in antipyrimidine and antipurine chemotherapy. Inhibitors of nucleoside transport (e.g. dipyridamole) and inhibitors of uridine kinase were evaluated for possible use in combination with inhibitors of de novo pyrimidine synthesis. Dipyridamole had only limited effectiveness in inhibiting nucleoside salvage in vivo, whereas 3-deazauridine was an effective inhibitor. Cyclopentenyl uracil, an effective inhibitor, was found to have many advantages over other compounds tested and will be studied in depth as supplies of this agent are made available.

We have recently developed a method, which is the only method available, to monitor the flux through the de novo pyrimidine pathway in vivo. $^{13}CO_2$ is administered to mice and the incorporation of ^{13}C into pyrimidine nucleotides is quantitated by GC/MS. Using this method we studied the effect of PALA, a potent inhibitor of de novo pyrimidine synthesis, on pathway flux in tumors sensitive and resistant to PALA. During the past year, this method was used to study the effect of acivicin, a glutamine analogue undergoing clinical evaluation, on de novo pyrimidine synthesis in L1210 (sensitive to acivicin) and B16 melanoma (resistant to acivicin) in vivo. The results of this study suggest that the effect of acivicin on carbamoyl-phosphate synthetase may be transitory and that the major effect of acivicin is due to inhibition of CTP-synthetase in the intact animal. GC/MS techniques were developed to quantify isotopic abundances in cytosine, cytidine, adenine, adenosine, quanine, guanosine, inosine, and hypoxanthine. These techniques will allow us to quantitate the flux through the de novo purine pathway in vivo simultaneous with measurements of the de novo pyrimidine pathway. We developed a method to quantify de novo pyrimidine biosynthesis based on the isotopic enrichment of N1 and N3 atoms of the uracil nucleus when animals are exposed to ^{15}N-NH_4Cl or ^{15}N-glutamine. From these values we are able to calculate, utilizing probability statistics, the number of uracil molecules produced by the de novo pathway that are labeled and those that are not labeled. By determining the enrichment of specific atoms of the product (uridine) formed in a pathway we know the isotopic enrichment of the immediate precursor pool for that atom. Comparison of this value with the precursor enrichment value obtained for the cell homogenate will indicate if compartmentation of precursors is occurring. We have developed a general mathematical framework using linear algebra that is implemented in a

computer program that will calculate the results of isotope dilution experiments for an arbitrary number of components. The generality of the framework has allowed us to develop a detailed mathematical treatment of biosynthetic studies which measure incorporation of a stable labeled precursor into a metabolic product. Feasability of the method was tested using ^{15}N-alanine in a time study in liver and intestine that showed the ratio of synthesis was linear with time. 1.5% and 7.5% of the uracil nucleotide pools contained in the liver and intestine, respectively were formed during a 1 h infusion of ^{15}N-alanine. A collaborative study was initiated that will use stable labeled precursors in man to study de novo purine synthesis in the bone marrow of patients receiving MTX.

The experience of investigators of this Laboratory in the pharmacology of antitumor agents was applied to several active antitumor agents under development by DTP for clinical trial. The pharmacokinetics of thioTEPA administered i.v. or intraventricularly was studied in monkeys and man. TEPA, a metabolite of thioTEPA which is known to be cytotoxic, was observed in all fluids and appeared to have a much slower total body clearance than thioTEPA. A method was developed to quantify dihydrolenperone, a cytotoxic agent with specificity for lung tumors which is currently under evaluation in man.

The preceding summary outlines the objectives of the Laboratory of Biological Chemistry and describes some of the research carried out within the Laboratory during the past year. The individual Project Reports, which follow, describe this research in greater detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06162-02 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacology of Antitumor Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.L. Cysyk	Chief	LBC, NCI
Others:	J.D. Moyer	Staff Fellow	LBC, NCI
	J.D. Strong	Sr. Investigator	LBC, NCI
	C.A. Chisena	Biologist	LBC, NCI

COOPERATING UNITS *(if any)*
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Clinical Pharmacology Branch, NCI (J. Collins)

LAB/BRANCH
Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	.5	.5

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☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cyclopentenyl cytosine, one of a new series of nucleoside analogues, was found to have marked in vivo antitumor activity in several murine models. It was especially active against an Ara-C resistant L1210 tumor. Inhibition of CTP synthesis was confirmed in mice bearing L1210 ascites. Cyclopentenyl-ara C and cyclopentenyl-8-aza-adenine were found to be cytotoxic to cultured L1210 cells. The pharmacokinetics of thioTEPA administered i.v. or intraventricularly was studied in monkeys and man. TEPA, a metabolite of thioTEPA which is known to be cytotoxic, was observed in all fluids and appeared to have a much slower total body clearance than thioTEPA. A method was developed to quantify dihydrolenperone, a cytotoxic agent with specificity for lung tumors which is currently under evaluation in man.

PHS 6040 (Rev. 1/84) GPO 814-918

Pharmacology of Cyclopentenyl Nucleosides

We are continuing to investigate a series of nucleoside analogs synthesized by the Laboratory of Medicinal Chemistry. The results with cyclopentenyl uracil, an inhibitor of uridine kinase, are described under project Z01 CM 06163-02. We have recently completed a study of the antitumor activity of cyclopentenyl cytosine, and presented our results to the Drug Evaluation Committee for consideration as a candidate for clinical trial.

We found that cyclopentenyl cytosine, a recently synthesized inhibitor of CTP synthesis, has marked anti-tumor activity. Treatment with 1 mg/kg i.p. on days 1-9 following inoculation with tumor produced 111-122% increased median life span (ILS) in mice bearing L1210 leukemia, 73-129% ILS in mice bearing P388 leukemia, and 58-62% ILS in mice with B16 melanoma. A subline of L1210 selected for resistance to cytosine arabinoside was more sensitive to cyclopentenyl cytosine than the parent tumor lines. L1210 cell growth in cultures was greatly inhibited (>90%) by 0.1 μM cyclopentenyl cytosine, but cells were protected from the growth inhibitory effects by cytidine (20 μM) and to a lesser extent by uridine or deoxycytidine. Exposure of cultured L1210 cells to 1 μM cyclopentenyl cytosine inhibited formation of [^{3}H]cytidine nucleotides from [^{3}H]uridine by 30% during the first 15 min of exposure to drug and by >95% after 2 h of exposure. Treatment of mice bearing L1210 ascites with 1 mg/kg cyclopentenyl cytosine produced rapid depletion of cytidine nucleotide pools in the tumor cells; these pools fell to 35% of control within 30 min. The effects of cyclopentenyl cytosine on nucleotide pools were tissue selective; the cytidine nucleotide pools of pleen, liver, kidney, and intestine were less sensitive than that of the L1210 ascites tumor. Cytidine nucleotide pools of spleen and liver were depleted by higher doses (10 mg/kg) of cyclopentenyl cytosine.

Further studies on the metabolism and mechanism of action of CPE-cytosine are in progress in collaboration with Drs. Kang and Cooney of the Laboratory of Experimental Therapeutics. Monophosphorylation of CPE-cytosine was catalyzed by L1210 cells as well as a partially purified preparation of uridine kinase with a K_m of 115 μM. CPE-cytosine also inhibited the phosphorylation of uridine competitively. Neither CPE-cytosine nor its monophosphate produced significant inhibition of CTP synthetase. However, exposure of L1210 cells to 100 μM CPE-cytosine resulted in formation of a component in acid-soluble extracts with the spectral and chromatographic properties expected for CPE-cytosine triphosphate at a concentration of 500 μM. This putative CPE-cytosine triphosphate is an inhibitor of CTP-synthetase with an IC_{50} of 2.5 μM. We suggest that this triphosphate is the active metabolite of CPE-cytosine.

Two additional cyclopentenyl nucleosides have been synthesized and tested for cytostatic activity in the last two months: cyclopentenyl-ara-C and cyclopentenyl-8-aza-adenine. These compounds inhibited L1210 cell growth in culture by 50% at 100 μM and 2 μM respectively. The marked cytotoxicity of CPE-8-aza-adenine will be examined in more detail. In particular we will determine the site of action by examining the effects of this agent on RNA synthesis, DNA synthesis, and nucleotide pools. Further studies of anti-tumor activity in vivo will be performed as drug is made available. Additional compounds will be synthesized and evaluated in the next year. We are particularly interested in the thymine derivative which we will evaluate as an inhibitor of thymidine kinase. Such an inhibitor may be of use in combination with methotrexate.

Pharmacology of Thiotepa. A study investigating the pharmacokinetics of intraventricular and intravenous administration of thiotepa in the rhesus monkey and man was recently completed and a manuscript submitted for publication. The objective of this study was to evaluate the relative merits of i.v. vs. i.t. administration of this drug for treatment of carcinomatous meningitis with this agent. After an i.v. dose of 0.9 mg/kg to monkeys, rapid equilibration of thiotepa concentrations in plasma, lumbar CSF, and ventricular CSF was most striking. Exposure to thiotepa, as measured by AUC values, was essentially equal for all 3 fluids. Persistence in the body is rather short (mean residence time, 50 min), and the elimination from the body is moderately fast (total body clearance, 37 ml/min/Kg). Thiotepa is rapidly removed from CSF following i.t. administration of 1 mg thiotepa to monkeys. The clearance of thiotepa from CSF (0.36 ml/min) exceeds the rate of bulk CSF outflow by approximately 8-fold. Although the i.t. dose was about 10-fold less than the i.v. dose, the AUC for ventricular CSF was nearly 100-fold greater for the i.t. route than for the i.v. route. However, the AUC for lumbar CSF following i.t. delivery was only 5% of the AUC for ventricular CSF. TEPA, a metabolite of thiotepa which is known to be cytotoxic, was observed in all fluids and appeared to have a much slower total body clearance. Comparison of the data obtained from the monkey experiments with data from a patient with meningeal disease supports the use of the monkey as a model for i.t. pharmacokinetics. The results of this study indicate that increased emphasis sould be given to systemic thiotepa treatment of CNS disease.

Pharmacology of Dihydrolenperone. Dihydrolenperone, a cytotoxic agent with known specific activity against certain lung tumors, is presently in clinical trials. We have developed an HPLC method to quantify plasma levels of this drug and are using the method for pharmacologic studies in conjunction with the phase I studies of dihydrolenperone. The method uses a C3-reverse phase column and heptane sulfonic acid as an ion pairing agent dissolved in a 70:30 acetonitrile:water as the mobile phase. Dihydrolenperone is extracted from plasma using a basic extraction into ethylacetate. After removal of the solvent, the residue is dissolved in 0.1 N HCL and injected into the HPLC for analysis. The minimum detection level is 0.1 ng with this and the minimum level for quantification of dihydrolenperone is 1 ng/ml plasma by this method. Analysis of serial plasma samples obtained from two patients after oral administration of the dihydrolenperone, revealed no plasma levels greater than 2 ng/ml. We are presently waiting for dose escalation to a level where plasma concentrations are high enough to perform pharmacokinetic studies.

Recently, we have started a collaboration with the Clinical Oncology Branch to help evaluate the effect of methotrexate on immature bone marrow cells. The objective of this study is to evaluate the effect of methotrexate on flux through the de novo purine biosynthetic pathway in these cells in vivo in human studies. We plan to use the stable isotope methods developed in our laboratory in combination with [^{15}N]-alanine as a precursor. The success of this project depends on the sensitivity of our techniques and we are presently testing the feasibility of this study in mouse bone marrow and with human bone marrow in vitro. The initial data we have collected looks positive. In one experiment mice were infused for 1 h with [^{15}N]-alanine and bone marrow, collected, pooled, and immature cells isolated. The cells were then analysed for ^{15}N-enrichment using our GC/MS method. The enrichment measured was 1-2 mole % and the precision of the measurement was acceptable.

Publications

1. Kang, G.J., Cooney, D.A., Moyer, J.D., Marquez, V.F. and Johns, D.G. Metabolic and mechanistic studies with cyclopentenyl cytosine. Proc. Am. Assoc. Cancer Res. 27: 297, 1986.

2. Moyer, J.D., Malinowski, N.M., Treanor, S.P., and Marquez, V.E. Antitumor activity and biochemical effects of cyclopentenyl cytosine in mice. Cancer Res. In press, July, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06163-02 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pharmacologic Aspects of Nucleotide Metabolism

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R. Cysyk	Chief	LBC, NCI	J. Strong	Pharmacologist LBC,NCI
Others:	L. Anderson	Chemist	LBC, NCI	D. Zaharevitz	Staff Fellow LBC,NCI
	S. Arnold	Chemist	LBC, NCI		
	O. Ayers	Lab. Technician	LBC, NCI		
	C. Chisena	Biologist	LBC, NCI		
	N. Malinowski	Chemist	LBC, NCI		
	J. Moyer	Sr. Staff Fellow	LBC, NCI		

COOPERATING UNITS *(if any)*
Medicinal Chemistry Section, LPET, NCI (V. Marquez)
Ohio State University (L. Malspeis)

LAB/BRANCH
Laboratory of Biological Chemistry

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The importance of intracellular purine salvage was examined. Adenine is salvaged from methylthioadenosine (MTA), a by-product of polyamine biosynthesis, in all normal cells and most tumor cells. It has been suggested that this salvage pathway be blocked in order to render the cells more sensitive to purine de novo synthesis inhibitors. Two cell lines, L1210 and L5178Y are similar in many parameters except the ability to salvage adenine from MTA. The basal rates of purine synthesis are similar in these two lines as is the sensitivity of the de novo pathways to 6-diaza-5-oxo-L-norleucine (DON). There is no difference in the ability of DON to inhibit growth in these two cell lines. Thus, from this limited study, developing blockers of salvage from MTA to use in combination with inhibitors of de novo purine synthesis is not a valid approach. Inhibitors of nucleoside transport (e.g. dipyridamole) and inhibitors of uridine kinase were evaluated for possible use in combination with inhibitors of de novo pyrimidine synthesis. Dipyridamole had only limited effectiveness in inhibiting nucleoside salvage in vivo, whereas 3-deazauridine was an effective inhibitor. Cyclopentenyl uracil, an effective inhibitor, was found to have many advantages over other compounds tested and will be studied in depth as supplies of this agent are made available.

Objective:

The overall objective of this project is to determine the relative dependency of host and tumorous tissues on de novo vs salvage pathways for the synthesis of pyrimidine and purine nucleotides in vivo. The de novo biosynthetic pathways supply pyrimidines and purines for nucleic acid synthesis and are therefore considered to be important pathways for cell proliferation. Therefore, there has been a considerable effort throughout the past several decades to develop specific inhibitors of enzymes of these pathways. Although a number of potent inhibitors (e.g. PALA, pyrazofurin, 6-azauridine) exhibit excellent in vitro activity against isolated enzymes and cultured cells and in vivo activity against certain murine tumors, only marginal clinical success has been achieved with these agents. It would appear that factors other than potency of inhibition are important because very potent enzyme inhibitors (PALA, pyrazofurin) are only marginally effective. Accordingly, lack of clinical success with the pathway inhibitors developed to date might be an indication that the importance of the de novo pathway to cell survival in vivo might be over-estimated. If so, then the development of additional inhibitors of the de novo pathway would be a futile effort unless there is a coordinate development of agents that either interfere with the salvage pathway or with the synthesis and export of preformed nucleosides by donor organ(s).

This Project is divided into the following specific aims: to determine the physiologic importance of circulating pyrimidines and purines and their role in modulating the antitumor activity of antipyrimidine and antipurine chemotherapeutic agents; to study the liver as a modulator of circulating nucleosides and as a possible target for chemical manipulation; to develop agents to interfere with nucleoside salvage to be used in combination with inhibitors of de novo synthesis; to develop methodology for monitoring and quantitating the flux through the de novo pathways of host and tumorous tissues in vivo.

Major Findings:

Development of Inhibitors of Nucleoside Salvage. The results discussed in previous annual reports of this project suggest that inhibition of nucleoside salvage could enhance the effectiveness of inhibitors of de novo nucleotide synthesis. Results we published this year have indicated that inhibitors of nucleoside transport, such as dipyridamole, have only limited potential as inhibitors of nucleoside salvage in vivo. However, this study also demonstrated, for the first time, an effective (>70%) inhibition of uridine salvage in vivo by 3-deazauridine, an inhibitor of uridine kinase. On the basis of these results and our recent survey of inhibitors of uridine kinase, we are examining the effect of cyclopentenyl uracil (CPE-uracil) on nucleoside salvage.

We examined the ability of CPE-uracil to inhibit uridine salvage by L1210 cells in culture and found that 100 μM CPE-uracil, a concentration without effects on cell replication, inhibited utilization of uridine by 80%. With this information we examined the use of CPE-uracil to increase the effectiveness of PALA, an inhibitor of pyrimidine synthesis de novo. PALA at 1 mM completely blocks cell growth in cultures, but if 5 μM uridine is added together with the PALA, cell growth is only reduced by 20%. Thus this low, near physiological concentration of uridine protects from the effects of PALA. If 100 μM CPE-uracil is also added to the cultures the PALA is fully effective

at inhibiting cell growth even in the presence of uridine. Therefore in this in vitro (culture) model CPE-uracil is an extremely useful drug in combination with PALA. We have now shown that CPE-uracil can also block uridine salvage in vivo. CPE-uracil pretreatment (1 g/kg) reduced salvage of uridine by various mouse tissues by 70-90% at 2 h after treatment. As more CPE-uracil becomes available we will further examine the inhibitory activity of this drug in vivo. In particular we will examine its duration of action and also extend our studies to other tissues and transplantable tumors. We will also do a study of the anti-tumor activity of CPE-uracil and PALA in combination against the murine P388 tumor model.

Purine Salvage. The importance of intracellular purine salvage was examined. Adenine is salvaged from methylthioadenosine (MTA), a by-product of polyamine biosynthesis, in all normal cells and most tumor cells. It has been suggested that this salvage pathway be blocked in order to render the cells more sensitive to purine de novo synthesis inhibitors. Two cell lines, L1210 and L5178Y are similar in many parameters except the ability to salvage adenine from MTA. The basal rates of purine synthesis are similar in these two lines as is the sensitivity of the de novo pathways to 6-diazo-5-oxo-L-norleucine (Don). If salvage from MTA is critical, the salvaged adenine would be able to circumvent the DON block of de novo purine synthesis and growth inhibition by DON be different in the two cell lines. However, there is no difference in the ability of DON to inhibit growth in these two cell lines. Thus, from this limited study, developing blockers of salvage from MTA to use in combination with inhibitors of the de novo purine synthesis in not a valid approach.

Publications

1. Moyer, J.D., Karle, J.M., Malinowski, N., Marquez, V.E., Salam, M.A., Malspeis, L., and Cysyk, R.L. Inhibition of uridine kinase and the salvage of uridine by modifieed pyrimidine nucleosides. Mol. Pharm. 28: 454-460, 1985.

2. Moyer, J.D., Malinowski, N., and Cysyk, R.L. The effect of 3-deazauridine and dipyridamole on uridine utilization by mice. Eur. J. Cancer Clin. Oncol. 22: 323-327, 1986.

3. Karle, J.M., Chisena, C., Cowan, K.H. and Cysyk, R.L.: Uracil nucleotide synthesis is a human breast cancer cell line (MC-7) and two drug resistant sublines that contain increased levels of enzymes of the de novo pyrimidine pathway. J. Mol. Pharm., in press, 1986.

4. Arnold, S.T. and Cysyk, R.L. Adenosine export from the liver: oxygen dependency. Am. J. Physiol., in press, 1986.

5. Klubes, P., Geffen, D.B. and Cysyk, R.L. Comparison of the bioavailability of uridine in mice after either oral or parenteral administration. Cancer Chem. and Pharm., in press, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06164-02 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibitors of Phospholipid Metabolism as Potential Chemotherapeutic Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Moyer	Staff Fellow	LBC, NCI
Others:	N. Dean	Visiting Fellow	LBC, NCI
	N. Malinowski	Chemist	LBC, NCI

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Biological Chemistry

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

Agents which block the formation of second messengers which mediate growth factor action may be of value in cancer chemotherapy. Recently the hydrolysis of phosphatidylinositol-diphosphate (PIP_2) to produce diacylglycerol (DAG) and inositol triphosphate (IP_3) has been implicated in the action of a number of growth factors, including platelet derived growth factor and bombesin. We are therefore attempting to examine the chemotherapeutic effects of inhibition of this process and to develop inhibitors of various enzymes involved in PI metabolism. During this period we have completed development of a new method for the separation and measurement of inositol phosphates. This technique has revealed that the metabolism of these compounds is more complex than previously known, and involves multiple positional isomers. Further studies are needed to explore the role of these compounds in growth factor action. Our initial efforts in drug development have centered on a series of myo-inositol analogs. We have developed a structure-activity profile for the incorporation of these analogs into fraudulent PI analogs. We also examined the uptake and utilization of myo-inositol and the new analogs by cells in culture.

Objectives. Reduced requirements for stimulation by growth factors may be the fundamental characteristic of transformed (neoplastic) cells. Research in molecular biology has identified several specific biochemical changes produced by introduction or overexpression of oncogenes which may reduce the levels of exogenous growth factors needed to trigger cell replication. These findings suggest that a more selective approach to chemotherapy may focus on the interaction of growth factors with cells rather than on basic metabolic reactions such as those involved in nucleic acid synthesis. We have therefore initiated a project to develop new chemotherapeutic agents to block the action of growth factors.

Recent findings indicate that inositol-phosphates formed from phosphatidylinositides may be the second-messengers which mediate the action of many growth factors. In addition, the product of two distinct oncogenes (src and ros) phosphorylate phosphatidylinositol and increase the levels of phosphatidylinositol polyphosphates in the membranes of cells transformed by these oncogenes. The synthesis of phosphatidylinositol polyphosphates and their subsequent hydrolysis therefore provide attractive well-defined targets for the design of inhibitors for use in chemotherapy. This project has two related goals. First, to determine the relative importance of the enzymatic reactions involved in inositol phosphate production as a signal initiating cell replication. Secondly, to design, synthesize, and evaluate drugs to inhibit this process, specifically inhibitors of the reactions of phosphatidylinositol polyphosphate synthesis and of phospholipase C, the enzyme which produces the inositol phosphates.

Major Findings. 1. A series of inositol analogs with single substitutions have been synthesized for us by Dr. David Baker of the University of Alabama. These analogs are potential inhibitors of the PI cycle, because they cannot be properly phosphorylated in that these compounds lack essential hydroxyl groups. Our studies have focused initially on the ability of the enzyme PI synthetase to incorporate these analogs into the corresponding analogs of PI. We found that any alteration at the 4 or 2 hydroxyl resulted in complete loss of ability to serve as a substrate for PI synthetase. In contrast, a number of compounds modified at the 5 position, notably 5-deoxy-myo-inositol and 5-deoxy-5-fluoro-myo-inositol were substrate for PI synthetase. Larger substituents in the 5 position eliminated substrate activity. Further study has shown that 5-deoxy-myo-inositol and 5-deoxy-5-fluoro-myo-inositol inhibit incorporation of myo-inositol into lipid by intact L1210 cells, presumably by competition. Furthermore, we have prepared [^{3}H]-5-deoxy-myo-inositol and found that it was incorporated into lipid both by extracts and intact L1210 cells.

2. Myo-inositol enters L1210 cells by a process not saturable to at least 5 mM, and reaches an intracellular concentration approximately equal to the external concentration.

3. A high performance liquid chromatographic separation was developed which resolves isomers of inositol monophosphate (IP), inositol biphosphate (IP_2) and inositol triphosphate (IP_3) in a single run. The system was used to investigate the thyrotropin releasing hormone (TRH) stimulated production of inositol phosphates in a rat pituitary tumor cell line (GH_3). In lithium treated cells prelabeled with [^{3}H]myo-inositol, radiolabeled inositol(1)phosphate (I(1)P), inositol(2)phosphate (I(2)P), inositol(4)phosphate (I(4)P), inositol

(1,4)bisphosphate, inositol (1,3,4)triphosphate ($I(1,3,4)P_3$) and inositol (1,4,5) triphosphate ($I(1,4,5)P_3$ are present. On exposure to TRH, both I(1)P and I(4)P increase, the increase in I(4)P preceeding that of I(1)P; $I(1,4)P_2$, $I(1,3,4)P_3$, and $I(1,4,5)P_3$ also increase. The increase in $I(1,4,5)P_3$ is rapid and transient while the increase in $I(1,3,4)P_3$ is slower and more sustained. The most rapidly appearing inositol phosphates produced subsequent to TRH stimulation are $I(1,4)P_2$ and $I(1,4,5)P_3$.

Proposed Course. 1. The effects of the newly prepared inositol analogs will be further explored to determine if they effectively prevent PI turnover. 2. New inhibitors of phospholipase C and PI kinase will be synthesized and evaluated. 3. The metabolism of the inositol phosphates in normal and transformed cells treated with growth factors will be examined in detail with the newly developed HPLC system. In collaboration with Dr. M. Beavin of the Heart Lung Institute we are examining the control of intracellular Ca^{++} levels by IP_3 in most cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06165-02 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Stable Isotope Studies of de Novo Pyrimidine and Purine Pathways

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	J. Strong	Pharmacologist	LBC, NCI
Others:	L.W. Anderson	Chemist	LBC, NCI
	D.W. Zaharevitz	Staff Fellow	LBC, NCI
	R.L. Cysyk	Pharmacologist	LBC, NCI

COOPERATING UNITS *(if any)*

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☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An existing stable isotope tracer methodology was used to monitor the flux through the de novo pyrimidine pathway in L1210-invaded spleens in vivo. The results of this study suggest that the effect of acivicin on carbamoyl-phosphate synthetase may be transitory and that the major effect of acivicin is due to inhibition of CTP-synthetase in the intact animal. Methodology was developed using GC/MS techniques in combination with HPLC separations for quantifying isotopic abundances in both purine and pyrimidine bases and nucleosides. A computer model using linear algebra techniques was developed which allows us to interpret data obtained from multiple stable label experiments. Studies were initiated using this methodology to determine the relative importance of the de novo pyrimidine and salvage biosynthetic pathways in normal and tumorous tissues in intact animals. Feasability of the method was tested using ^{15}N-alanine, a time study in liver and intestine that showed the ratio of synthesis was linear with time. 1.5% and 7.5% of the uracil nucleotide pools contained in the liver and intestine, respectively were formed during a 1 h infusion of ^{15}N-alanine.

PHS 6040 (Rev. 1/84) GPO 914-918

Radiolabeled tracer methodology has played a major role in the elucidation of metabolic pathways. Such information, coupled with specific activity determinations of enzymes in cell-free extracts, is a valuable indicator of the importance of a particular pathway in the intact cell. Studies designed to measure pathway activity need to be carried out in intact cells because of the improbability of duplicating, in vitro, the intracellular microenvironment in which the enzyme functions. Moreover, the concept of intracellular compartmentation of pathway activities has now replaced the simplistic view of the cell as a bag of uniformly dispersed enzymes acting on a homogeneous supply of metabolites. Intracellular and intercellular compartmentation of enzymes and small molecules necessitates experimentation with intact cells and animals, however at the same time, complicates the interpretation of results obtained using tracer methodology. The use of stable labeled compounds as tracers combined with mass spectrometric techniques in some cases are more advantageous for these types of studies. Precursors can be labeled with the appropriate stable isotope to essentially 100% enrichment without posing a biohazard. These high enrichments minimize the dilution effects which occur especially in vivo. Stable isotopes such as nitrogen and oxygen which have no radiolabel isotopes easily available can be used as tracers and most importantly, these techniques are easily adapted to studies where several atoms in the product molecule are labeled. The latter advantage will allow us to address some of the problems with tracer studies such as the fact that isotope enrichment determinations in pathway products following administration of labeled precursors represent an average enrichment value which includes (a) isotopically enriched product molecules that were formed from the labeled pathway under study and (b) non-isotopically enriched product molecules that were either present prior to the labeling period or were produced by an alternate pathway during the course of the labeling experiment. A method which would distinguish a-type molecules from b-type molecules would be a desirable technique to study pathway activity and regulation. Furthermore, such a method would be valuable in compartmentation studies since the enrichment of the newly synthesized product molecules would be a measure of the isotopic enrichment of the precursor molecules in the cellular compartment from which the product is derived.

Objective

The objective of this project is two-fold: (1) to develop stable-label methodology to quantitate the flux through the de novo pyrimidine and purine pathways in vivo and in intact cells in vitro, and (2) to develop stable-label methodology and computer models to study compartmentation of reaction pathways in vivo and in intact cells in vitro.

Measurement of flux through the de novo pyrimidine and purine pathways. The stable isotope $^{13}CO_2$ method previously developed (Strong et.al. Anal. Biochem. 132: 243-253, 1983) used to monitor flux through the de novo pyrimidine pathway was employed to study the effect of acivicin on L1210-invaded spleens (a new tumor model developed in this laboratory). Acivicin, a glutamine analog which acts as an irreversible enzyme inhibitor is presently undergoing clinical investigations. A controversy presently exists as to the relative importance of its inhibitor effect on two enzymes; carbamoyl-phosphate synthetase and CTP-synthetase. Using the $^{13}CO_2$ method, flux through the de novo pyrimidine pathway in the L1210-invaded spleens was determined in mice pretreated with acivicin. These measurements, combined with the determination of uracil

nucleotide pools and cytidine nucleotide pools by HPLC have resulted, to date, in the following observations: 2 h after pretreatment with acivicin, the uracil nucleotide pool was expanded to 140% and the cytidine nucleotide pool decreased to 80% of control values. Four hr after treatment, the uracil nucleotide pool returned to control values and remained constant up to 24 h after pretreatment. In contrast, the cytidine nucleotide pool continued to decrease and was 50% control values 24 h after pretreatment with acivicin. Flux through the de novo pyrimidine pathway was maximally inhibited <10% control values) 2 h after treatment and the pathway returned to its full complement by 24 h.

This data suggests that the effect of acivicin on carbamoyl-phosphate synthetase may be transitory and the major effect of acivicin is due to inhibition of CTP-synthetase in the intact animal. A method has been developed to quantify the incorporation of ^{13}C into cytidine. Using this method and combined treatments with acivicin and PALA, which inhibits ATCase enzyme in the pyrimidine pathway, the relative effects and time course of inhibition of the two enzymes by acivicin will be determined. In conjuction with these studies, the effects of acivicin on the purine pathways are being investigated. In addition, the $^{13}CO_2$ method will continue to be used to investigate the effects of new antitumor agents which are designed or expected to inhibit the de novo biosynthetic pyrimidine pathway in intact animals.

Although methods have been developed to quantify incorporations of stable isotopes into the purine nucleotide pools in vivo, these techniques only measure the over all ^{15}N isotopic enrichments in the purine bases. Attempts are presently being investigated to allow isotope enrichment determinations for the individual nitrogens in the purines. These values will be important for other studies in the laboratory.

Pharmacologic considerations of pathway compartmentation. Recently we reported a method to quantify de novo pyrimidine biosynthesis based on the isotopic enrichment of N1 and N3 atoms of the uracil nucleus when hepatocytes are exposed to $^{15}NH_4Cl$ or ^{15}N-glutamine, (Strong, et.al, J. Biol. Chem. 260: 4276-4281, 1985). The information we are able to extract from a stable isotope experiment of this type where at least 2 atoms in a product molecule are enriched by stable labeled precursor molecules is unique. A mathematical model and a computer program recently written, is used to determine the specific enrichment of the immediate precursor molecules of the product and the fraction of the product pool that was derived from the labeled pathway during the course of the experiment. With this information, the absolute amount of product formed, and the absolute rate of pathway activity can be determined. The enrichment determined in the immediate precursor pool can be compared to the precursor enrichment measured in the total cellular pool. Based on this type of information, intracellular or intercellular comparmentation of pathway intermediates can be evaluated.

The pyrimidine and purine nucleotide pools can be supplied by either de novo or salvage pathways. The relative importance of the two alternate sources of these nucleotides has been of interest to the laboratory because of the use of antitumor agents which act to effect these pathways. The objective of this project is to determine the relative importance of the pyrimidine and purine salvage and de novo biosynthetic pathways in normal and tumorous tissues in intact animals and in clinical studies using our stable isotope methodology.

Long term studies in mice (7 day s.c. infusions) were initiated to evaluate the labeling pattern in pyridines and purines contained in various tissues by $^{15}NH_4Cl$, ^{15}N-glycine, ^{15}N-alanine, and ^{13}C-acetate. The latter ^{13}C labeled compound was of interest for tracing the source of the carbon backbone of the pyrimidines, purines, and their ribose sugar. We were primarily interested in the multiple labeling characteristics of the stable isotope precursors which would allow us to analyse the data by our unique method discussed. The results of these experiments indicated that low dose $^{15}NH_4Cl$ (50 μMoles/day) was not very effective for dilabeling of pyrimidines, but primarily labels N3 in the uracil molecule. At higher doses (250 μMoles/day), however, this compound is very effective at dilabeling both the pyrimidine and purine bases. Based on equimolar amounts, the other precursors were compared to that observed for $^{15}NH_4Cl$. ^{15}N-Glycine was as effective for labeling purines but showed less incorporation into pyrimidines than that derived from $^{15}NH_4Cl$. ^{15}N-Alanine was an excellent precursor for multiple labeling both purines and pyrimidines. This precursor produced essentially equal atom % enrichments in the N1 and N3 positions of uracil which is optimum for our multiple label analysis method. ^{13}C-Acetate was not very effective under these conditions for labeling either purines or pyrimidines.

Studies have been initiated using ^{15}N-alanine in combinations with phosphorylase enzymes and specific kinase inhibitors to determine the rates of salvage vs. de novo pyrimidine nucleotide biosynthesis. Short term i.p. administration of ^{15}N-alanine to mice (0.5 h, 1 h, and 2 h) and a comparison of the measured rate of synthesis by the de novo pyrimidine pathway in liver and intestines resulted in the following observations: The time study showed for both tissues, that the fraction of the uracil nucleotide pool produced from the de novo pathway was linear with time. 1.5% of the total uracil nucleotide pool contained in the liver and 7.5% of the uracil nucleotide pool in intestine were formed during a 1 h infusion experiment. Absolute rates of 22 pmole/h-g wet wt. and 60 pmol/h-g wt. were calculated for liver and intestine, respectively. We are interested in which organs may produce preformed nucleosides for export to tumor tissue as wll as the rates of salvage and de novo synthesis and feel the stable isotope methodology we have developed will allow us to perform these types of studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06166-02 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanism of Multidrug Resistance

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, leboretory, and institute effiliation)*

PI:	Ronald L. Felsted	Research Chemist	LBC, NCI
Others:	Ahmad R. Safa	Visiting Fellow	LBC, NCI
	Constance Glover	Microbiologist	LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.7	1.8	.9

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the spece provided.)*

Radioactive photoactive drug analogues have been used to identify specific drug binding targets in multidrug-resistant cells. A 150-180 kDalt surface membrane glycoprotein (gp150-180) has been identified in drug-resistant but not in drug-sensitive parental cells. Gp150-180 exhibits cross binding specificity for Vinca alkaloids, actinomycin D, anthracycline antibiotics, calcium channel blockers and calmodulin inhibitors suggesting a central functional role in the multidrug-resistant phenotype. The role of gp150-180 in drug-resistance will be tested by examining the effect of specific drug photolabeling on cellular drug uptake and efflux. The subcellular distribution of gp150-180 will be determined. A functional gp150-180 will be purified. Anti-gp150-180 monoclonal antibodies will be prepared and gp150-180 plasma membrane orientation with respect to drug binding sites will be determined. Partial amino acid sequence analysis will be used to construct specific gp150-180 cDNA probes. Purified gp150-180 will be inserted into planar lipid bilayers for functional reconstitution studies. The biochemical role(s) of gp150-180 in drug resistance will be studied in order to design new agents for circumventing the multidrug-resistant phenomenon.

GPO 814-918

The exposure of malignant cell lines to natural product cytotoxic drugs such as vinblastine, actinomycin D, adriamycin or colchicine frequently results in the isolation of populations of cells with resistance to the selecting agent as well as a collateral resistance to other mechanistically distinct and structurally unrelated compounds. The mechanism(s) by which these cell lines become multi-drug-resistant is unknown, but it is thought to be related to a parallel reduction in the cellular accumulation of those drugs to which the cells are resistant. The multidrug-resistant phenotype also is characterized by the presence of a 150-180 kDa surface membrane glycoprotein (gp150-180) which occurs in multidrug-resistant cells in direct proportion to the degree of their acquired drug-resistance. The relationship of gp150-180 to multi-drug resistance is not known. It may accumulate only as a secondary consequence of the multidrug-resistant phenotype. Alternatively, gp150-180 could promote multi-drug resistance by direct or indirect effects on membrane permeability, drug transport, or drug binding.

Objectives.

This project involves the identification of specific drug interactions with macromolecules in normal and multidrug-resistant cell lines. The relationship of specific drug binding macromolecules to multidrug-resistance mechanisms will be examined. New chemotherapeutic agents designed to circumvent multidrug-resistance will be designed, synthesized and tested.

Major Findings.

Identification and characterization of anthracycline acceptors in drug sensitive- and drug-resistant P388 murine leukemic cell line. N-(p-Azidobenzoyl)-daunorubicin, ([^{3}H]NABD), a radioactive photoactive anthracycline analog was used to photoaffinity label anthracycline binding polypeptides in P388 murine leukemic cell lines. Either intact cells or cell homogenates were mixed with 6×10^{-8} M [^{3}H]NABD, exposed to UV-light and analyzed by SDS-polyacrylamide gel electrophoresis for [^{3}H] incorporation. Autoradiofluorography showed incorporation of radioactivity into a M_r 18,000 component independent of polypeptides prominently stained with Coomassie blue. Photolabeling of subcellular fractions showed predominant mitochondrial localization of the M_r 18,000 radiolabel. The protein composition of the photolabeled constituents was confirmed by treatment with proteinase K, DNase and RNase, or by lipid extraction with organic solvent. Photolabeling with increasing concentrations of [^{3}H]NABD resulted in a biphasic pattern of radiolabel incorporation suggesting that both specific and nonspecific binding to the M_r 18,000 was present. [^{3}H]NABD photolabeling of homogenates from anthracycline sensitive (P388/S) and resistant (P388/DRN) cells resulted in M_r 18,000 radiolabel incorporation of 3966 ± 355 dpm and 6487 ± 533 dpm per 50 μg cellular protein for P388/S and P388/DRN cells, respectively ($p<.005$). These studies characterize the photoaffinity labeling of a low molecular weight mitochondrial polypeptide using a photoactive anthracycline analog. The role for this polypeptide as a mediator of anthracycline activity remains to be determined.

Leukemic cells Vinca alkaloid acceptors. We have synthesized a photoactive radioactive analog of vinblastine (VB), N-[p-azidobenzoyl]-N'-β-aminoethyl-vindesine (NABV), in order to evaluate the interactions of Vinca alkaloids with cellular macromolecules. [^{3}H]-NABV was used to photoaffinity label Vinca

alkaloid binding components (VABCs) in P388 murine leukemic cell homogenates. After irradiation at 302 nm, VABCs were resolved by SDS-PAGE and identified in 1 mm gel slices as radioactive labeled components. The most prominent photolabeled species were M_r 54 and M_r 44 Kd components located in the 100,000xg supernatant fraction. M_r 54 was also observed in the particulate fraction. VABCs were not affected by DNase or RNase. Extraction with chloroform:methanol (2:1) revealed no [^{3}H]-labeled lipid macromolecules. However, proteinase K treatment reduced radiolabeling to the ambient background level, confirming a polypeptide composition for all VABCs. The M_r 54 Kd was identified as a tubulin subunit by immunoprecipitation with anti-tubulin monoclonal antibody. The tubulin subunits were photolabeled in a ratio of 3:2 (α:β). The M_r 44 polypeptide was not immunoprecipitated with antitubulin or anti-actin antibodies and remains unidentified. After 72 hr exposure, the IC_{50} values of exponentially growing P388 leukemic cells were 1.1 nM for NABV and 0.6 nM for VB. The ultrastructural effects of NABV and VB on P388 cells were similar: formation of microtubular crystals; mitotic arrest (C-mitosis); increased post C-mitotic multinucleated cells; increased annulated lamellae; the appearance of intracytoplasmic paired cisternae; and increased number of intermediate bundles. Thus, NABV is an attractive tool for identification and characterization of cellular acceptors which initiate or mediate both known and novel mechanisms of *Vinca* alkaloid action.

Tubulin Vinca alkaloid binding sites. We have synthesized a pharmacologically active, photoactive, radioactive analog of vinblastine (VB), N-(*p*-azido-[3,5-^{3}H]benzoyl)-*N*'-β-aminoethylvindesine (NABV) and used it to probe *Vinca* alkaloid macromolecular interactions. In the present study we have used NABV to photolabel purified calf brain tubulin. The polymerization of 15 μM tubulin in the presence of microtubule-associated proteins was inhibited by 50% at 2 μM NABV or VB. At concentrations higher than 5 μM, both NABV and VB induced tubulin aggregation *in vitro* and in P388 murine leukemic cells, and both drugs inhibited tubulin-dependent GTP hydrolysis. Under equilibrium conditions 10 μM NABV or VB reduced the binding of 10 μM [^{3}H]-VB tubulin by about 50%. Mixtures of tubulin (3 μM) and [^{3}H]-NABV (0-24 μM) were irradiated at 302 nm for 20 min at 25°C, subjected to SDS-PAGE, and incorporation of radioactivity into protein was quantitated. Photolabeling of both the α and β subunits of tubulin with increasing concentrations of [^{3}H]-NABV exhibited a biphasic pattern characteristic of specific and nonspecific photolabeling. Nonspecific labeling was determined in the presence of 200-fold excess of VB. After correcting for nonspecific labeling, saturable covalent incorporation was found in both subunits of tubulin in an α:β ratio of 3:2. Photolabeling of the tubulin subunits will permit localization of *Vinca* alkaloid binding sites, which is essential for understanding the interaction of tubulin with these drugs and their effects on microtubule assembly.

Vinca alkaloid acceptors in multidrug-resistant cells. Photoactive radioactive analogues of vinblastine were used to photoaffinity label membranes of Chinese hamster lung drug-sensitive (DC-3F), multidrug-resistant sublines selected for resistance to vincristine (DC-3F/VCRd-5L) or actinomycin D (DC-3F/ADX), and revertant (DC-3F/ADX-U) cells. A radiolabeled doublet (150-180 kDa) consisting of a major and minor band which was barely detectable in parental drug-sensitive cells was increased up to 150-fold in the drug-resistant variants but only 15-fold in the revertant cells. Photoaffinity labeling in the presence of 200-fold excess vinblastine reduced radiolabeling of the 150-180 kDa species up

to 96% confirming its Vinca alkaloid binding specificity. The radiolabeled doublet comigrated with a Coomassie blue stained polypeptide doublet in the drug-resistant cells and was immunoprecipitated with polyclonal antibody which is specific for the 150-180 kDa surface membrane glycoprotein (gp150-180) in multidrug-resistant cell lines. The identification of this Vinca alkaloid acceptor in multidrug-resistant plasma cell membranes suggests the possibility of a direct functional role for gp150-180 in the development of multi-drug resistance.

Proposed Course.

The above data confirm the usefulness for identifying drug binding proteins by photoaffinigy labeling. A major gp150-180 Vinca alkaloid acceptor has been identified in multidrug-resistant cells. Other drugs will be used to establish the multidrug specificity of gp150-180. The role of gp150-180 in drug resistance will be examined using agents previously shown to counteract multidrug-resistance. The effect of photolabeling on cellular drug uptake and efflux will be examined. The subcellular distribution of gp150-180 with time will be monitored. The gp150-180 will be purified using immunoabsorption and affinity chromatographic methods. Purified gp150-180 will be used to prepare monoclonal antibodies and structurally define plasma membrane orientation and drug binding sites. Partial structural analysis will be used to construct genetic probes. Ultimately, gp150-180 will be inserted into planar lipid bilayers for functional reconstitution experiments. From this understanding of the multidrug-resistance mechanism, we will synthesize and test new compounds designed to reverse the multidrug-resistant state.

Publications:

1. Felsted, R.L., Gupta, S.K., Glover, C.J. and Gallagher, R.E. Surface membrane glycoproteins of wild-type and differentiation-inducer-resistant HL-60 cells. Blood 66: 606-613, 1985.

2. Averbuch, S.D., Clawson, R.E., Bachur, N.R. and Felsted, R.L. Cellular pharmacology and antitumor activity of N-(p-azidobenzoyl)daunorubicin, a photoactive anthracycline analogue. Cancer Chemother. Pharmacol., 1986, in press.

3. Safa, A.R., Glover, C.J., Meyers, M.B. Biedler, J.L. and Felsted, R.L. Vinblastine photoaffinity labeling of a high molecular weight surface membrane glycoprotein specific for multidrug-resistant cells. J. Biol. Chem., 1986, in press.

4. Averbuch, S.D., Glover, C.J. and Felsted, R.L. Anthracycline photoaffinity labeling of a mitochondrial polypeptide in P388 murine leukemic cell lines. Cancer Res., 1986, in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06167-02 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Inhibition of Myristoylation-Dependent Oncogene Mediated Cellular Transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	R.L. Felsted	Research Chemist	LBC, NCI
Other:	C. Glover	Microbiologist	LBC, NCI
	C. Goddard	Visiting Fellow	LBC, NCI

COOPERATING UNITS *(if any)*
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LAB/BRANCH
Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The modification of onc-proteins with the fatty acid myristate is an early step associated with the transformation of normal to neoplastic cells. The exact significance of myristoylation in transformation has not been established. However, it is thought to be part of the mechanism by which cytoplasmic oncogene kinases are localized to the inner plasma membrane surface. Since the transforming activity of onc-kinases is dependent upon this membrane binding, this project will investigate the role of myristoylation as it relates to the mechanism of this subcellular localization. A specific myristoylation assay is being developed using synthetic NH_2-terminal polypeptide homologues of cellular and viral myristoylated proteins. The myristyl transferase(s) substrate specificity for polypeptide and fatty acid will be examined in order to define the myristoylation mechanism. The involvement of myristic acid in membrane binding will be examined by looking for specific membrane receptors of the myristoyl-proteins. Information on the enzymology of myristoylation and the role of myristic acid in translocation and membrane binding will be used to design and synthesize specific inhibitors of myristoylation in these related phenomena with the goal of developing chemotherapeutic agents specific for a critical early steps of tyrosine kinase mediated malignant transformation.

Tyrosine-specific protein kinase activity is associated with several known oncogenes and is an appealing target for the chemical manipulation of kinase associated cellular transformation. Selective inhibition of onc-kinases appears to be a formidable task, but may become possible when the cellular substrate(s) for the onc-kinases are identified. Recently it has been observed that direct inhibition of the onc-kinases may not be necessary in order to block (or reverse) transformation. The transforming activity of onc-kinases depends upon their association with the inner plasma membrane surface. It has been proposed that the mechanism by which viral encoded onc-kinases such as p60src become membrane bound is through a post- or co-translational addition of myristic acid to their NH_2-terminal glycine via an amide linkage. In recently published experiments, Cross et al. constructed p60src NH_2-terminal deletion mutants that failed to incorporate myristic acid. These mutant proteins were still functional kinases in vitro and in vivo, but instead of being localized to the membrane they were found in the cytoplasm. Most pertinent to this proposal, these mutant proteins no longer transformed cells. Presumably, myristoylation is critical to p60src subcellular localization and cellular transforming activity.

Myristoylation is also necessary for the membrane localization of the cellular p60src tyrosine kinase encoded by the src proto-oncogene. In addition, several normal and neoplastic mammalian cells have elevated levels of a similar size tyrosine kinase, p58. However, this p58 kinase is distinct from the cellular and viral p60src as well as from five other proto-oncogene cellular tyrosine kinases and it does not contain gag linked viral proteins. Most interestingly, one of these p58 kinases contains myristic acid. These results indicate that myristoylation may be an important regulator of the subcellular distribution and function of normal cellular tyrosine kinases. Thus, transformation resulting from the expression of structurally modified tyrosine kinases or elevated amounts of cellular kinases also may depend on myristoylation-directed subcellular distribution.

Objectives.

In this project we will examine the role of myristoylation of onc-tyrosine kinases as targets for the chemotherapeutic inhibition of cellular transformation. Specifically, we will study three mechanisms for blocking myristoylation dependent transformation by p60src: these include, (i) inhibition of the myristoyl transferase(s), (ii) the blocking of translocation of soluble myristoyl-proteins to their membrane binding sites, and (iii) inhibition of binding of myristoyl-proteins to potential membrane receptors. We will examine, also, the relationship of myristoylation to normal tyrosine kinases and the role of myristoylated-tyrosine kinases in normal cellular growth and differentiation.

Major Findings and Proposed Course

i. Myristoyl Transferase(s). Fatty acid amides are well known in nature and their syntheses by eucaryotic cell free systems have been described. Myristoyl amidation takes place during or immediately after protein synthesis so fatty acid addition may occur by a co-translational process as in the case for NH_2-terminal acetylation. The myristoyl transferase(s) also are characterized by an absolute specificity for myristic acid. The question of substrate specifi-

city will be examined in order to develop a specific enzyme assay for myristoylation. It is likely that nascent polypeptide chains are the actual substrates of the myristoyl transferase(s). Studies with p29gag-ras and deletion construct mutants of p60src indicate that the first 13-15 terminal amino acids may present a conformation-dependent myristoylation acceptor site as the substrate for myristoyl transferase(s). We have synthesized a 15 amino acid polypeptide corresponding to the NH_2-terminal sequence of the p60src protein. In addition, a tyrosine residue was added to the COOH-terminal end of the synthetic polypeptide to allow for radiolabeling with ^{125}I. Both N-myristoylated and NH_2-terminal glycine ^{125}I labeled peptides have been purified and are readily resolved by TLC and HPLC. The NH_2-terminal glycine peptide will be tested as a substrate in a specific myristoyl transferase assay and used as a probe for myristoyl-protein acceptors (see ii and iii, below).

Synthetic polypeptides corresponding to several known myristoyl acceptor amino acid sequences will be tested to assess relative myristoyl transferase substrate specificity. Polypeptide analogues of varying lengths and with selected amino acid substitutions will be tested as competitive inhibitors. In collaboration with Dr. Victor Marquez of the Laboratory of Medicinal Chemistry and Pharmacology, DTP, DCT, NCI, we will design and synthesize polypeptide and/or fatty acid derivitives that will serve as specific irreversible (suicide) inhibitors of the myristoyl transferases(s). Also, once the fatty acid myristoyl donor has been identified, it might be possible to block myristoylation by inhibiting the de novo synthesis of the fatty acid donor.

ii. Myristoyl-protein translocation. Shortly after synthesis on free ribosomes, the myristoyl-p60src kinase forms a soluble complex with two cellular proteins of 50 and 90 kilodaltons. It has been suggested that this complex may act as a device for transporting newly synthesized p60src kinase to its site of transforming action at the plasma membrane. Similar complexes have been reported for onc-kinases encoded by other retroviruses so "complex mediated" translocation might be another important step in onc-kinase cellular transformation. Although the p50 and p90 proteins are not thought to bind to the NH_2-terminal region of p60src, the role of myristoylation in this complex is not understood. It may be possible to establish the function of myristic acid in the carrier mediated translocation by testing to see if mutants of p60src kinases which lack myristic acid are still able to form comparable complexes with p50 and p90. Ultimately, it may be necessary to use specific inhibitors of myristoyl transferases(s) (see i, above) or myristoyl-protein binding (see iii, below) in order to clarify the role of myristic acid in translocation.

iii. Myristoyl-protein membrane binding. Myristic acid may play a direct role in the binding of onc-proteins to the plasma membrane. This could occur by one of two basic mechanisms. In one mechanism, the hydrophobic fatty acid could serve as an anchor for the protein by embedding into the membrane lipid bilayer. In fact, this has been shown for some integral membrane proteins where the palmitic acid esterification of membrane bound nascent polypeptides is part of a maturing process which results in these proteins being more firmly anchored in the membrane. This anchor mechanism also is compatible with the carrier mediated translocation process described above (see ii.). Since myristoylation occurs in the cytoplasm, premature anchoring to the nearest intracellar membrane bilayer could be prevented by complexing the newly modified

onc-protein to specific carrier proteins. In turn the carrier proteins could provide the recognition signal that determines the ultimate membrane destination. However, once the complex arrives at the membrane and the carrier proteins release the transported onc-protein, membrane binding must be determined by intrinsic structural features of the myristoyl-onc-protein; in this case, myristate insertion into the membrane bilayer. The problem with this hypothesis is that in contrast to the irreversible binding of palmitoyl ester modified integral membrane proteins, myristoyl-proteins are reversibly membrane bound.

A second mechanism which could provide for reversibility would be the recognition of and binding to myristate by specific membrane receptors. If, in addition, the receptor recognizes the adjoining NH_2-terminal polypeptide sequence, then an added degree of specificity would result (i.e. the binding of p60src specifically to membrane adhesion plaques). We are examining the role of myristoyl-protein binding by classical receptor techniques. Using the radiolabeled ^{125}I-labled myristoylated-synthetic polypeptide described above (see i.), we have demonstrated saturable binding to human red cell membrane vesicles. Analysis suggest high and low affinity classes of binding sites, both of which are competed with unlabeled myristoylated peptide but not by unlabeled NH-terminal glycine peptide. This displacement assay will be used to survey for potential inhibitors of myristoyl-protein receptor binding.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06168-02 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Pharmacology of Interferon, Double-Stranded RNA and Growth Factors

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Marian Knode	Biologist	LBC, NCI
	Mrunal Chapekar	Visiting Associate	LBC, NCI
	Ives Pommier	Visiting Associate	LMP, NCI
	Eugene Tilchen	Clinical Associate	LMP, NCI

COOPERATING UNITS *(if eny)*

Laboratory of Molecular Pharmacology, DTP, DCT, NCI (I. Pommier and E. Tilchen)

LAB/BRANCH

Laboratory of Biological Chemistry

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NCI, NIH, Bethesda, Maryland 20892

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The antiprolferative and synergistic effects of immune interferon (IFN-γ) and the synthetic double-stranded RNA (dsRNA), poly(I)·poly(C), on human colon carcinoma cell lines HT-29 and BE were examined in vitro. HT-29 cells which are sensitive to the cytocidal effects of IFN-γ show a synergistic antiproliferative response to IFN-γ and poly(I)·poly(C), the latter of which is noncytotoxic. BE cells which are resistant to IFN-γ are sensitized in the presence of poly(I)·poly(C). The synergistic effects of IFN-γ and poly(I)·poly(C) were related to inhibition of transcription of rRNA in HT-29 cells but not in BE cells and were unrelated to the IFN-γ-mediated induction of 2',5'(oligo)adenylate synthetase in either cell line. In other studies, poly(I)·poly(C) administered i.v. to mice bearing the L1210 leukemia transplanted s.c., produced an increase in the therapeutic index of 5-fluorouracil or 5-fluorouridine were administered i.v. and concurrently with poly(I)·poly(C). These effects correlated with an increased IFN production and associated 2',5'(oligo)A synthetase activity in the bone marrow and spleen, implying a cytoprotective effect by endogeneously secreted IFN. Current studies are examining the synergism between poly(I)·poly(C) and epidermal growth factor (EGF) in the dsRNA-sensitive cell line A431, a human epidermoid carcinoma containing an amplification in the EGF receptor gene. The proliferative response of a clone of A431 cells to EGF is being studied with respect to its response to EGF and poly(I)·poly(C) and the activity of topoisomerase II and its associated tyrosine kinase activity.

The mechanism of action of interferon (IFN) and double-stranded RNA (dsRNA) has been an area of investigation in this laboratory for the past 5 years. Initial studies sought to examine the role of the dsRNA-dependent 2',5'(oligo)adenylate synthetase in the antiproliferative activity of IFNs and the use of 2',5'oligoadenylate analogs as possible mediators of IFN activity. It was apparent from investigations of several human tumor cell lines that the 2',5'(oligo)A pathway was not mediating the antiproliferative effects of IFN-α, -β and -γ, and that despite the induction of 2',5'(oligo)A synthetase by the various IFNs, no measurable amounts of 2',5'(oligo)A could be detected by a radioligand assay. Moreover, 2',5'(oligo)A analogs of cordycepin and xylosyladenine served as prodrugs of the cytotoxic nucleoside and not as mediators of 2',5'(oligo)A-dependent processes such as the 2',5'(oligo)A-activated ribonuclease. Despite these results, it was apparent that the dsRNA, poly(I)·poly(C) produced dramatic potentiation of the cytocidal effects of immune IFN (IFN-γ) against human colon carcinoma cells in vitro. Poly(I)·poly(C) (which is not cytotoxic) produced a synergistic cytocidal effect with IFN-γ against HT-29 cells. In human colon carcinoma cell line BE which is inherently resistant to IFN-γ, poly(I)·poly(C) (which is not cytotoxic), as well as several other synthetic dsRNAs sensitized the cells to the cytocidal activity of IFN-γ. In HT-29 cells, inhibition of rRNA synthesis correlated with cytocidal activity but this effect was not apparent in BE cells. Thus, mechanistically, the sensitizing effect of dsRNAs as well as the pharmacological action of IFN-γ remain unknown. Recently, we have discovered that human epidermod carcinoma A431 is inherently sensitive to the cytotoxicity of dsRNAs and may serve as a good model system to examine the mechanism of action of dsRNA. Interestingly, this cell line contains an amplification as well as a mutation in the epidermal growth factor (EGF) receptor gene. We have recently found that not only are dsRNAs cytotoxic in this cell line but that synergistic growth inhibition is obtained with very low levels of EGF and dsRNA. These results correlated with the reduction of tyrosine kinase activity, ie. EGF receptor tyrosine kinase, and suggests that dsRNA may be exerting its effect at the level of the EGF receptor. Studies underway are examining EGF binding in the presence of dsRNA, as well as autophosphorylation of the EGF receptor.

Recent evidence suggests that the proliferative effect of EGF may be associated with increased topoisomerase II activity. The activity of topoisomerase II can be inhibited in vitro by purified tyrosine kinase. The activity of topoisomerase II in response to the growth inhibitory effects of EGF are not known. Similarly, the role of tyrosine kinase regulation of topoisomerase II and the associated stimulatory or inhibitory activities of EGF is unknown. Thus, A431 cells appear to represent an excellent model by which to investigate the growth regulatory properties of EGF and the associated functions of tyrosine kinase and topoisomerase. By this approach, we plan to use two clones of A431 cells (Gordon Gill's lab. UCSD) which either proliferate or are growth inhibited in response to EGF, and to analyze topoisomerase II and EGF receptor and nuclear tyrosine kinase activities in collaboration with Ives Pommier and Eugene Tilchen, Laboratory of Molecular Pharmacology. Toward this end we have found tyrosine kinase activity to be tightly associated with the nuclear matrix complex of topoisomerase II in L1210 cells.

Major Findings:

1. The effect of poly(I)·poly(C) on the antitumor activity of 5-fluorouracil

(FUra) and 5-fluorouridine (FUrd) was evaluated in mice bearing L1210 leukemia. Coadministration i.v. of poly(I)·poly(C) and either FUra or FUrd on days 1,5, and 9 to mice bearing L1210 leukemia implanted s.c resulted in a 40% greater increase in life span at the optimal antitumor dose versus FUra and FUrd alone. This effect appeared to result from greater host tolerance of a dose of FUra or FUrd which would otherwise be cytotoxic. The protective effect of poly(I)·poly(C) was also evident in nontumor-bearing mice, as well as following administration of drug i.p. to mice bearing the tumor implanted i.p. FUrd incorporation into RNA in the spleen, bone marrow, and small intestine revealed little or no changes after coadministration of poly(I)·poly(C). (2',5')Oligo(A) synthetase activity, an indication of IFN secretion, was markedly depressed in the spleen and bone marrow following treatment with FUrd; however, poly(I)·poly(C) administered together with FUrd returned (2',5')oligo(A) synthetase activity to normal levels. These data indicate that poly(I)·poly(C) ameliorates the host toxicity of fluoropyrimidines, possibly via an IFN-mediated effect, and thereby results in enhanced therapeutic efficacy of the antimetabolites as antitumor agents.

2. The cytocidal activity of human IFN-γ in combination with poly(I)·poly(C), was investigated in human colon carcinoma cell line HT-29. Three day treatment with 10 to 25 units/ml of IFN-γ resulted in 30 to 40 % reduction in colony formation, whereas 25 to 100 μg/ml of poly(I)·poly(C) reduced cell viability by 10 to 20% of control. The lethal effect of the combination of IFN-γ and poly(I)·poly(C) was synergistic wherein 70 to 90% reduction in colony formation was observed. Measurements of DNA, RNA and protein synthesis after IFN-γ and poly(I)·poly(C) treatment showed a dose-dependent reduction in all three parameters. Recombinant IFN-γ in combination with poly(I)·poly(C) exhibited a similar effect. Studies evaluating the molecular mechanism of IFN-γ and poly(I)·poly(C) toxicity indicate a lack of involvement of the dsRNA-dependent (2',5')oligo(A)-RNAse L and protein kinase pathways; however, the effect appears to be related to the inhibition of ribosomal RNA transcription in this cell line.

3. A human cell line BE, derived from an undifferentiated carcinoma of the colon, was studied for its response to the cytocidal effects of human IFN-γ alone, and in combination with various dsRNAs. BE cells were moderately refractory to three day treatment with IFN-γ (10 to 300 units/ml) where only a 5 to 30% reduction in colony formation occurred. A similar exposure interval to 100 μg/ml of poly(I)·poly(C) had no detectable effect on colony formation. In contrast, the lethal effect of the combination of IFN-γ and poly(I)·poly(C) was synergistic and this regimen produced a 40 to 80% reduction in colony formation. The cytocidal effects of the combination of IFN-γ with varying concentrations of the dsRNAs poly(I)·poly(C), poly(A)·poly(U), poly(ICLC) and $rI_n \cdot r(C_{13},U)_n$ were also examined. The concentration of the dsRNAs producing a 50% decrease in cell viability in combination with 100 units/ml of IFN-γ, was 6 μg/ml for poly(I)·poly(C), 1 μg/ml for poly(A)·poly(U), 3 ng/ml for poly(ICLC), and 16 μg/ml for $rI_n \cdot r(C_{13},U)_n$. DNA, RNA, and protein synthesis in IFN-γ and poly(I)·poly(C)-treated cells were reduced in a dose-dependent manner. However there were no changes in either (2',5')oligo(A) concentrations or in rRNA transcription following treatment with IFN- and poly(I) poly(C).

Thus, the synergism resulting from the combination of IFN-γ and dsRNA appears to be mediated via another, as yet unknown, mechanism.

4. The growth inhibitory effects of varying concentrations of the dsRNAs, poly(I)•poly(C), poly(A)•poly(U), poly(ICLC), and $rI_n \cdot r(C_{13},U)_n$ were investigated in human epidermoid carcinoma cell line A431 which has an amplification in the EGF receptor gene. The concentrations of the dsRNAs producing a 40% decrease in cell growth after 3 days of treatment was 20 μg/ml for poly(I)•poly(C), 100 μg/ml for poly(A)•poly(U), 0.2 μg/ml for poly(ICLC), and 40 μg/ml for $rI_n \cdot r(C_{13},U)_n$. Measurement of DNA and RNA synthesis after treatment with dsRNA showed a dose-dependent reduction in these parameters. One or 3 day treatment of A431 cells with either poly(I)•poly(C), poly(A)•poly(U), poly(ICLC), or $rI_n \cdot r(C_{13},U)_n$ failed to induce (2',5')oligo(A) synthetase activity indicating that the observed growth inhibition was not related to IFN production by these dsRNAs. The antiproliferative effect of poly(I)•poly(C) was investigated in A431 cell variants. Clone 29 which proliferates in response to EGF and clone 29R which is sensitive to the growth inhibitory effects of EGF showed similar sensitivities to the growth inhibitory effects of poly(I)•poly(C). Growth inhibition in the parent A431 cell line by the combination of poly(I)•poly(C) with varying concentrations of EGF was also examined. Poly(I)•poly(C) at 2.5 μg/ml reduced cell growth by 25% and 1 to 100 ng/ml of EGF resulted in 25 to 40% growth inhibition after 3 days of treatment. Poly(I)•poly(C) in combination with EGF produced a potentiation of growth inhibition. The relationship between cytotoxicity and EGF receptor tyrosine kinase as well as the activity of topoisomerase II and its associated tyrosine kinase is under investigation.

Publications

1. Iigo, M., Chapekar, M.S. and Glazer, R.I.: Synergistic antitumor effect of fluoropyrimidines and polyinosinic-polycytidylic acid against L1210 leukemia. Cancer Res. 45: 4039-4042, 1985.

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3. Chapekar, M.S. and Glazer, R.I.: Cell viability and the (2',5')oligo(A)-RNase L pathway in human colon carcinoma cells treated with human inter ferons and double-stranded RNA. In Williams, B.R.G. and Silverman, R.H. (Eds): 2,5A System: Molecular and Clinical Aspects of the Interferon Regulated Pathway. Proceedings of the 6th International Symposium of the Research Institute, Hospital for Sick Children. New York, NY, Alan H. Liss, 1985, pp. 377-384.

4. Chapekar, M.S. and Glazer, R.I.: Potentiation of the cytocidal effect of human immune interferon by different double-stranded RNAs in the refractory human colon carcinoma cell line BE. Cancer Res. 46: 1698-1702, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06179-01 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Role of Protein Kinase C & Tyrosine Kinases in Differentiation and Drug Resistance

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Mruanl Chapekar	Visiting Associate	LBC, NCI
	Angelo Aquino	Guest Worker	LBC, NCI
	Yu Gang	Visiting Fellow	LBC, NCI
	Kathleen Hartman	Chemist	LBC, NCI
	Marion C. Knode	Biologist	LBC, NCI
	Kenneth Cowan	Senior Investigator	CPB, NCI
	Ronald Felsted	Senior Investigator	LBC, NCI
COOPERATING UNITS *(if any)*	Ahmad Safa	IPA	LBC, NCI

Clinical Pharmacology Branch, DCT, NCI
J. F. Kuo, Professor, Emory University
Steven Grant, Assistant Professor, Columbia University

LAB/BRANCH
Laboratory of Biological Chemistry

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SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Calcium- and phospholipid-dependent protein kinase (PK-C) activity was determined in association with the differentiation response of HL-60 leukemia cells to DMSO, retinoic acid, 1,25-dihydroxyvitamin D_3, phorbol ester (TPA) and interferon-γ. PK-C activity rose in proportion to the appearance of the mature granulocytic or monocytic phenotype. Conditions under which TPA produced the macrophage phenotype resulted in disappearance of PK-C, but duplication of this phenotypic response with one hour priming doses of TPA and post treatment with retinoic acid showed conclusively that down regulation of PK-C is not a necessary consequence of the macrophage phenotype. A new investigation has been initiated to study the regulation of plasma membrane-associated tyrosine kinase in HL-60 leukemia cells undergoing differentiation and in cells resistant to differentiating agents. In addition, the relationship between membrane tyrosine kinase and pleiotropic drug resistance in human breast carcinoma cell line MCF-7 is being investigated to assess the association between the p170 glycoprotein induced in multidrug resistant cells, calcium channels and tyrosine kinase. To investigate these problems, a new nondenaturing gel electrophoretic assay for multiple tyrosine kinase activities in cell extracts has been developed.

The role of protein kinases in the regulation of cell proliferation and differentiation is receiving increased emphasis within the framework of recent advances in protooncogene function and hormone and growth factor receptors. The calcium- and phospholipid-dependent protein kinase (protein kinase C, PK-C) which is ubiquitous within the animal kingdom is believed to serve a crucial regulatory role in mediating or modulating the action of growth factors. For example, phosphorylation of EGF receptor by PK-C reduces the receptors affinity for EGF. The human promyelocytic leukemia cell line HL-60 contains an abundance of PK-C. We have recently documented increased PK-C in response to granuloctic or monocytic differentiating agents such as DMSO, retinoic acid and 1,25-dihydroxyvitamin D_3. Interferon-γ (IFN-γ) and tumor necrosis factor (TNF), both discovered in our lab to be monocytic differentiating agents, also induce elevated PK-C activity in the mature phenotype. The response of PK-C to these various differentiating agents appears to be differentiation-related and serves as a good marker of the mature phenotype. Recently, we have found a rapid redistribution of PK-C in response to IFN-γ as assessed by immunocytochemical methods in collaboration with J.F. Kuo, Emory University. The implications of these experiments are that an early membrane response of HL-60 cells to differentiating agents may be the plasma membane association of PK-C and subsequent phosphorylation events.

An ancillary area of research involves our recent discovery of a plasma membrane-bound tyrosine kinase that is induced during the differentiation of HL-60 cells by several granulocytic (DMSO, retinoic acid) and monocytic (1,25-dihydroxyvitamin D_3, tetradecanoyl phorbol acetate, IFN-γ and TNF differentiating agents. This tyrosine kinase appears within 1 day after treatment of HL-60 cells with IFN-γ or TNF and is elevated in proportion to the degree of maturation of the cells. The development of a non-denaturing polyacrylamide gel electrophoretic assay system has allowed us to characterize the various species of tyrosine kinase activities in crude cytoplasmic and membrane preparations in a variety of cell lines including drug resistant cells. We have recently found in collaboration with Steven Grant, Columbia University, that HL-60 cells that are 100-fold resistant to Adriamycin are cross resistant to several differentiating agents and are devoid of the membrane-bound tyrosine kinase found in HL-60 cells sensitive to these agents.

In other studies in collaboration with Ken Cowan, Clinical Pharmacology Branch, NCI, MCF-7 cells showing the multidrug resistance phenotype, also possessed large amounts of a membrane-bound tyrosine kinase which was absent in the wild type cells. The possible association of this activity with the drug resistance glycoprotein, p170, is currently being investigated in collaboration with Ronald Felsted and Ahmad Safa, LBC. Thus, one of the more promising avenues of study will entail purifying and characterizing the physiological role of the tyrosine kinases associated with differentiation and drug resistance. If p170 is found to be associated with tyrosine kinase then modulators of this activity may have therapeutic potential for modifying multidrug resistant tumor cells.

In addition, the role of nuclear tyrosine kinase in the regulation of topoisomerase II and the effects of EGF upon topoisomerase II activity in response to the proliferative or growth inhibitory effects of EGF is being evaluated (see project "Cellular Pharmacology of Interferon, Double-Stranded RNA and Growth Factors" in collaboration with Ives Pommier and Eugene Tilchen, LMP.

Major Findings

1. The effects of differentiating agents on the activity and phosphorylation pattern produced by PK-C were examined in human promyelocytic leukemia cell line HL-60. Dimethyl sulfoxide (DMSO), retinoic acid (RA) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) increased the appearance of mature myelocytic (DMSO and RA) or monocytic ($1,25(OH)_2D_3$) cells. The tumor promoter, 12-O-tetradecanoylphorbol-13-acetate (TPA) increased the appearance of adherent macrophage-like cells. Coincident with the appearance of differentiated cells induced by DMSO, RA and $1,25(OH)_2D_3$ was an increase in PK-C activity. In contrast, TPA treatment resulted in the rapid disappearance of PK-C and the induction of phospholipid and Ca^{++}-independent protein kinase activity. The phosphorylation pattern resulting from endogenous PK-C in extracts from cells treated with DMSO, RA or $1,25(OH)_2D_3$ showed a prominent phosphorylated protein of 37 kilodaltons (pp37) and 38 kilodaltons (pp38) which was related to the appearance of the myelocyte/monocyte phenotype. pp37 and pp38 were also present in TPA-treated cells but their phosphorylation was no longer dependent on the presence of phospholipid and Ca^{++}. Cells treated with DMSO and RA also exhibited a phospholipid- and Ca^{++}-dependent pp21 which was barely evident in $1,25(OH)D_3$-treated cells, and thus, represented a myeloid cell marker. Also present was a prominent phospholipid- and Ca^{++}-dependent pp19 which remained unchanged following treatment with DMSO, RA and $1,25(OH)_2D_3$, but was markedly diminished in TPA-treated cells. On the other hand, TPA-treated cells exhibited a characteristic pp130 which was antigenically related to the actin-binding protein, vinculin. These results indicate that there are characteristic phospholipid- and Ca^{++}-dependent phosphorylated proteins indicative of mature myelocytic and monocytic cells, as well as phospholipid and Ca^{++}-independent phosphorylated proteins characteristic of the macrophage-like phenotype produced by TPA.

2. The ability of retinoic acid (RA) to promote TPA-initiated macrophage differentiation was examined in human promyelocytic leukemia cell line HL-60. One hr exposure to 10 nM TPA and subsequent exposure for 48 hr to 1 μM RA following removal of TPA rapidly induced the macrophage phenotype in 65% of the cells. This effect was comparable to continuous exposure for 48 hr to TPA alone, but contrasted with the absence of macrophage-like cells after RA treatment alone or the induction of 10% of the cell population to a macrophage phenotype after 1 hr exposure to TPA. The effect of TPA + RA was accompanied by increased cell adherence and increased nonspecific esterase activity but not by a change in the reduction of nitroblue tetrazolium. Protein kinase C (PK-C) activity was increased 35-40% in cells treated for 1 hr with TPA alone or after subsequent exposure to RA. Cells treated for 48 hr with RA exhibited a 2-fold increase in PK-C activity while cells exposed to TPA for 48 hr lost all PK-C activity. The changes in PK-C activity in TPA + RA-treated cells were accompanied by phospholipid- and Ca^{++}-dependent phosphorylation *in vitro* of pp38 which is characteristic of treatment with RA alone, as well as the phospholipid- and Ca^{++}-independent phosphorylation *in vitro* of pp82 and pp130 (vinculin) which is prevalent in cells treated continuously with TPA alone and is absent in RA-treated cells. These results indicate that the macrophage phenotype induced by TPA + RA is similar to that produced by continuous exposure to TPA alone with respect to their *in vitro* phosphoprotein patterns, cytochemical

markers, cell adherence and morphology, but that the disappearance of PK-C is not an obligatory characteristic of these cells.

3. The effect of the combination of RA and calcium ionophore A23187 on cellular differentiation was assessed in promyelocytic leukemia cell line HL-60. RA at 10^{-10} to 2.5 x 10^{-8} M or 4 x 10^{-7} M A23187 produced a 15 to 22% increase in differentiated cells reducing nitroblue tetrazolium. Exposure of cells for 48 h to the combination of 4 x 10^{-7} M A23187 and 10^{-10} to 2.5 x 10^{-8} M RA resulted in a 20 to 86% increase in cells reducing nitroblue tetrazolium, but with no measurable level of nonspecific esterase activity. Cotreatment of these cells with A23187 and either DMSO, 1,25dihydroxyvitamin D_3 or human immune IFN-γ failed to produce a synergistic effect on differentiation. Addition of either the calmodulin antagonists, N-(6-aminohexyl)-5-chloronaphthalenesulfonamide (W7) and trifluoperazine, or the protein kinase C inhibitor, 1-(5-isoquinolinesulfonyl)-2-methylpiperazine (H-7) during treatment with A23187 and RA did not block differentiation. Membrane tyrosine kinase activity was measured in cells treated with A23187 and RA in a nondenaturing gel system using the exogenous substrate poly(Glu:Tyr). Membrane-bound tyrosine kinase activity was not present in untreated or RA-treated cells, but was induced by A23187 treatment alone and was markedly increased in cells after 48 h treatment by the combination of A23187 and RA. Significant reduction in c-myc mRNA levels was also observed after 24 h treatment of HL-60 cells with RA and A23187 but not by either agent alone. These results suggest that a Ca^{2+} mediated process sensitizes cells to the differentiating effect of RA and that this effect is associated with a significant reduction of c-myc expression and the induction of membrane tyrosine kinase activity in this cell line.

4. The effect of IFN-γ and recombinant tumor necrosis factor (rTNF-α) on cellular differentiation was investigated in human promyelocytic leukemia cell line HL-60. Both IFN-γ and rTNF-α induced the appearance of the monocytic phenotype in a dose- and time-dependent manner as assessed by morphology, reduction of nitroblue tetrazolium and the induction of nonspecific esterase. Utilizing a nondenaturing polyacrylamide gel electrophoretic assay, it was revealed that a membrane-bound tyrosine kinase activity accompanied the appearance of the differentiated cell type. In contrast, other tyrosine kinase activities were either unaltered or reduced during differentiation. These results suggest that the induction of membrane-bound tyrosine kinase by IFN-γ and rTNF-α may be an important determinant of the differentiation process.

Publications

1. Zylber-Katz, E. and Glazer, R.I.: Phospholipid- and calcium-dependent protein kinase activity and protein phosphorylation in human promyelocytic leukemia cell line HL-60. Cancer Res. 45: 5159-5164, 1985.

2. Zylber-Katz, E., Knode, M.C. and Glazer, R.I.: Retinoic acid promotes phorbol ester-initiated macrophage differentiation in HL-60 leukemia cells without disappearance of protein kinase C. Leukemia Res., in press, 1986.

3. Glazer, R.I.: Differentiation of malignant cells as a new mode of chemotherapy. In Elsebai, I. (Ed.): Current Treatment of Cancer. Heidelberg, UICC-Springer-Verlag, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06180-01 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
The Sulfhydryl Group in Cancer Cell Growth, Metastasis and Chemotherapy

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Marco Rabinovitz Research Chemist LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biological Chemistry

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SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The homocysteine derivative, S-methylthio-DL-homocysteine (SMETH) was synthesized and found to be cytotoxic to L1210 cells in culture. Its cytotoxicity was increased in the presence of leucine, which interferes with glutamine utilization as an energy source. A working hypothesis is presented which describes the interaction of homocysteine, formed by intracellular disulfide reduction of SMETH, with adenosine, formed by intracellular dephosphorylation of ATP. This mechanism may be applicable for development of chemotherapy of slowly growing or latent tumors.

We had earlier indicated that the thiol requirement for L1210 cells in primary culture can also be met by the addition of bathocuproine sulfonate, a copper-specific chelator. This sensitivity to trace copper has subsequently been found to be also a property of human lymphomas, and bathocuproine sulfonate is reported to be a required additive for the establishment of new human lymphoma cell lines. The oxidative stress introduced by copper can also be overcome by using 4-thiamethionine, the already oxidized mixed disulfide of methyl mercaptan and cysteine, as a source of cysteine. This compound, a methionine analog, is taken up by the cells via the leucine transport system and reduced intracellularly to cysteine to satisfy the requirement for this amino acid. The derivatization of cysteine with the methylthio- group to prevent oxidative dimerization and promote uptake for growth can serve as a model for potentiation of the activity of cytotoxic and chemotherapeutic sulfur and selenium compounds.

Homocystine is not actively transported by cells since its two additional methylene groups beyond that of cystine make it an unsuitable substrate for the cystine-glutamate carrier. It is proposed that uptake of homocysteine by cells be promoted as its S-methylthio- derivative; which should be actively transported via an amino acid transport system and reduced intracellularly to homocysteine. In the cell, depending upon the availability of adenosine, homocysteine may be converted to S-adenosylhomocysteine (SAH) via the enzyme SAH hydrolase. S-Adenosylhomocysteine is a potent inhibitor of cellular methylations and has anti-viral and anti-neoplastic properties, but is not readily taken up by cells.

Objectives

The general goals of this project are directed toward an understanding of the factors influencing the sulfhydryl-disulfide status of the cell and cellular milieu, and the application of this knowledge in the development of selectively toxic regimens for chemotherapy. This portion of the project is focused on an improved method for delivery of homocysteine to cells via its methylthio-derivative and introduction of mechanisms for modulating its cytotoxicity by metabolic alterations which may be applicable to chemotherapy.

Major Findings

S-Methylthio-DL-homocysteine (SMETH) was synthesized. This derivative of homocysteine is designed to promote the uptake of homocysteine via one of the aliphatic amino acid transport systems. It was cytotoxic in the micromolar range to L1210 cells in culture and promoted cell lysis at higher concentrations (200 μM).

The potency of cytotoxic amino acids, (e.g. melphalan) can be reduced by retarding their uptake through competition with a natural amino acid, (e.g. leucine). Leucine, however, markedly promoted the cytotoxicity of SMETH. Analysis of this phenomenon indicated that leucine interfered with the utilization of glutamine, a critical substrate for energy metabolism.

The current working hypothesis is that formation of adenosine is promoted when adenosine triphosphate cannot be maintained by energy generating processes and that such adenosine functions as co-substrate with homocysteine for the enzyme, SAH hydrolase, to form SAH. This putative mechanism would be unique for

metabolic inhibitors, which normally require maintenance of adenosine triphosphate to promote kinase activities and macromolecular syntheses. It could be especially effective in development of regimens against slowly growing or latent tumors.

Proposed Course

1. The working hypothesis described above will be checked by measurement of cellular constituents and by inhibition analysis of participating enzymes.

2. Synergism between ATP depleting regimens and SMETH will be evaluated, and those regimens least toxic to the host will be used in combination for *in vivo* chemotherapeutic evaluation.

3. These studies will be extended to human tumor cells in culture and to xenografts.

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1. Fisher, J.M. and Rabinovitz, M.: Selenocystine is not a cystine antimetabolite in L1210 cells. Cancer Lett. 28: 43-46, 1985.

2. Mohindru, A., Fisher, J.M. and Rabinovitz, M.: Endogenous copper is cytotoxic to a lymphoma in primary culture which requires thiols for growth. Experientia 41: 1064-1066, 1985.

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4. Pierson, H.F., Fisher, J.M. and Rabinovitz, M.: Modulation by taurine of the toxicity of taumustine, a compound with antitumor activity. JNCI: 75: 905-909, 1985.

5. Pierson, H.F., Fisher, J.M. and Rabinovitz, M.: Methylthio-capping of selenocysteamine: preliminary studies on selective toxicity for cancer chemotherapy. Res. Commun. Chem. Path. Pharmacol.: 50: 447-450, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06181-01 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*

Interaction of GTP-Binding Membrane Proteins with Cellular Components

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Neme, title, laboretory, end institute effilietion)*

PI: Richard A. Kahn Senior Staff Fellow LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

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☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Activation of the ras oncogene has been implicated as the causative agent in as many as 30% of all human tumors. Yet in spite of extensive work on the ras gene, including the identification of at least seven different ras genes and an increasing number of ras-like genes, almost nothing is known about the function of the ras proteins in higher eukaryotes. A recently characterized protein, termed ARF, is a component of the adenylate cyclase system and shares several features with ras, including size, location, and ability to bind GTP. Characterization of the binding and hydrolysis of guanine nucleoties by ras and ARF will be undertaken. A systematic search will then be conducted to identify factor(s) which increase either the exchange or hydrolysis of guanine nucleotides by these regulatory proteins. These studies should locate cellular targets for these proteins and may identify the physiological role of these proteins in cellular metabolism or proliferation.

Research on the regulation of the adenylate cyclase system has led to the discovery of a family of homologous GTP-binding, regulatory proteins (G-proteins). Five distinct members have been identified and purified, though only two have defined roles in the regulation of adenylate cyclase activity. Although implicated as regulators of K^+, Ca^{++} and other ion channels, phosphoinositide metabolism, and cellular proliferation, the roles of the other G-proteins are not yet known.

A number of recent techniques in molecular biology have led to the discovery of another family of GTP-binding membrane proteins which are products of the ras oncogene. Seven distinct ras genes have been identified. Ras proteins have been shown to activate adenylate cyclase from one strain of yeast but have no known function or role in any higher eukaryotes.

A novel G-protein has been purified and characterized by the PI. This protein, termed ARF, interacts with the adenylate cyclase system and shares many of the GTP-binding characteristics of ras. Both proteins have a molecular mass of 21,000 daltons.

Objectives

In all cases where a cellular role is known the activity of a guanine nucleotide binding protein is controlled by the binding of GTP. Hydrolysis of the bound GTP results in deactivation. Thus, knowledge of the factors which control the binding and hydrolysis of GTP will also identify elements upstream and downstream of the regulatory proteins. Thus, the objective of this work will be to utilize the nucleotide binding properties of these two proteins, ras and ARF, to identify cellular targets and pathways controlled by either of these proteins.

Methods employed

The principal method utilized will be radioligand binding studies of nucleotide binding to purified ARF and ras proteins. Factors which increase either the off-rate of bound GDP or hydrolysis of bound GTP will be screened for in tissue extracts. Standard protein purification methods will be utilized to further characterize any activity present.

Major findings

The half-time of GDP release from ARF is 24 min under certain conditions. This can be increased more than 10-fold ($t_{1/2} \approx 2$ min) by the addition of a detergent extract of bovine brain membranes. The factor(s) responsible for this change in affinity of ARF for GDP is under investigation.

Publications

1. Kahn, R.A. and Gilman, A.G. A family of guanine nucleotide binding proteins: transducers of membrane signaling. In Paton, W., Mitchell, I. and Turner, P. (Eds.): Proceedings of the Ninth International Congress of Pharmacology, MacMillan Press Ltd., 1984, pp. 287-291.

2. Kahn, R.A. and Gilman, A.G. The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP-bindign protein. J. Biol. Chem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06182-01 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Genetic and Immunologic Analysis of a Novel Regulatory Membrane Protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Richard A. Kahn Senior Staff Fellow LBC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biological Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1	.5	.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The identification of ARF, a component of the adenylate cyclase system, as a GTP-binding regulatory protein is of considerable interest due to the similarities between ARF and the products of the ras oncogene (molecular mass, membrane location, GTP binding characteristics). In addition, expression of ras proteins has profound effects on cyclic nucleotide levels in some systems. ARF was purified from bovine brain membranes. Cyanogen bromide fragments of the pure protein were separated and two amino acid sequences of about 25 residues each were obtained. Synthetic peptides were synthesized for injection into rabbits to produce specific antibodies to ARF. Oligonucleotide probes were also synthesized from the amino acid sequence and are being used to screen a cDNA library to obtain the full length sequence of ARF. These antibody and cDNA probes should help to identify the site and physiological role of this novel membrane protein. Comparison of the primary and predicted tertiary structure of ARF to other recently cloned G-proteins may aid in the identification of specific domains involved in binding other proteins or binding and hydrolyzing GTP. In this case, site-directed mutagenesis will be used to construct specific altered proteins to test for cellular functions of ARF.

PHS 6040 (Rev. 1/84) GPO 914-918

ARF has recently been identified as a 21,000 Da GTP-binding regulatory membrane proteins. When activated ARF binds to the stimulatory, regulatory component (Gs) of adenylate cyclase and allows cholera toxin to irreversibly activate the cyclase. Thus, though apparently a component of the adenylate cyclase complex, the physiological role of ARF is unknown. ARF is present in the plasma membrane of every eukaryotic tissue or cell type examined. Attempts to isolate ARF-deficient mutant cell lines have been unsuccessful in a number of different laboratories. ARF has the same molecular mass, cellular localization, and shares some of the GTP binding characteristics with products of the ras oncogene. There are also clear differences between these proteins, e.g. no immunological cross-reactivity. Ras proteins have been implicated in changes in cyclic nucleotide levels in several tissues and have recently been shown to directly stimulate adenylate cyclase in one strain of yeast. The role of ras proteins in adenylate cyclase of higher eukaryotes is unknown but is clearly not a direct coupling to the cyclase.

Objective

The objective of this work is the cDNA cloning of ARF and comparison to ras. In addition, monospecific peptide and protein antibodies to ARF will be produced to further characterize the cellular location and requirement for ARF.

Methods Employed

The methods employed will include the screening of a cDNA library with oligonucleotide probes to obtain a full length cDNA coding for ARF followed by the nucleotide sequencing of the cDNA and comparison to other known sequences. The library being screened is an Okiyama-Berg plasmid vector with bovine adrenal chromaffin cDNAs. Synthetic peptides will be coupled to carrier and injected into rabbits to produce monospecific antibodies against ARF. The synthetic peptides will also be used to affinity purify ARF-directed antibodies. The presence of ARF in a cell or tissue will be detected by Western blotting procedures. Cellular localization of ARF will require immunocytochemistry. If proper probes, e.g. inactivating antibodies, or altered ARF constructs, are devised; then microinjection of these probes into cells in culture will be examined as a means of detecting new activities for ARF.

Major Findings.

A new method for the purification of ARF from bovine brain allowed the generation of sufficient protein for amino acid sequencing. Cyanogen bromide fragments of ARF were sequenced and yielded two stretches of about 25 residues each. Each fragment contained sequences previously identified in other GTP-binding proteins as highly conserved residue involved in the binding of guanine nucleotides. Peptides to each of these regions were synthesized for the production of antibodies to ARF. It is hoped that these antibodies may bind to the nucleotide binding site and prevent activation. Oligonucleotide probes were also synthesized and are currently being used to screen a cDNA library for full length ARF message.

Proposed Course

1. Clone ARF and compare the primary sequence to ras and other GTP-binding proteins. Use site directed mutagenesis to create altered forms of ARF at specific residues. Express the clones or altered ARF genes in normal cells.

2. Produce high titer monospecific antibodies to ARF. Determine if any alter ARF function when bound. Microinject inactivating ARF antibodies and/or altered ARF constructs into normal cells.

Publications

1. Kahn, R.A. and Gilman, A.G. A family of guanine nucleotide binding proteins: transducers of membrane signaling. In Paton, W., Mitchell, I. and Turner, P. (Eds.): Proceedings of the Ninth International Congress of Pharmacology, MacMillan Press Ltd., 1984, pp. 287-291.

2. Mumby, S.M., Kahn, R.A., Manning, D.R., and Gilman, A.G. Antisera of designed specificity for subunits of guanine nucleotide-binding regulatory proteins. Proc. Natl. Acad. Sci. U.S.A. 83: 265-269, 1986.

3. Kahn, R.A. and Gilman, A.G. The protein cofactor necessary for ADP-ribosylation of Gs by cholera toxin is itself a GTP-binding protein. J. Biol. Chem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07109-10 LBC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Cellular Pharmacology of Nucleoside Analogs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Robert I. Glazer	Senior Investigator	LBC, NCI
Others:	Kathleen D. Hartman	Chemist	LBC, NCI
	Marian C. Knode	Biologist	LBC, NCI
	Kasturi Sriram	Visiting Fellow	LBC, NCI
	Victor E. Marquez	Visiting Scientist	LPET, NCI

COOPERATING UNITS *(if any)*

Medicinal Chemistry Section, LPET, DTP, DCT, NCI (V. Marquez)

LAB/BRANCH

Laboratory of Biological Chemistry

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.5	1.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

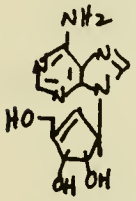
☐ (a1) Minors

☐ (a2) Interviews

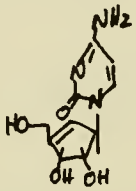
SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

The mechanism of action of nucleoside analogs was investigated in human carcinoma cell line HT-29 and human promyelocytic leukemia cell line HL-60. Cyclopentenyl adenosine (neplanocin A) was an effective inhibitor of RNA and DNA methylation in HL-60 cells via its metabolism to an S-adenosylmethionine (AdoMet)-like metabolite. However, neplanocin proved to be a poor inducer of differentiation in HL-60 cells as assessed by nitroblue tetrazolium reduction and cellular myc RNA expression. The 3-deaza analog of neplanocin, c^3Nep, was as effective as an inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase as neplanocin but possessed 1/10 the cytotoxicity, did not effectively inhibit RNA methylation and was not metabolized to an AdoMet-like metabolite. c^3Nep was ineffective as a differentiating agent in HL-60 cells. The cyclopentenyl cytidine analog (cCyd) possessed a potent inhibitory effect on CTP synthesis and subsequently, DNA synthesis with moderate cytotoxicity against HT-29 and HL-60 cells. However, this agent proved to be a very effective differentiating agent for HL-60 cells, probably as a result of its S phase specific effects at noncytotoxic concentrations. In other studies, the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), was found to produce complete remissions in hairy cell leukemia in phase II clinical trials. Experimental studies in mouse lymphocytes indicated that the antileukemic effect of dCF may be a result of inhibition of lymphokine synthesis, viz. IL-2 and immune interferon, which are known to be growth factors for hairy cell (B-cell) leukemia cells.

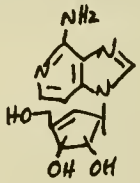
Molecular and Cellular Pharmacology of Nucleoside Analogs. Studies over the past year have involved our continuing collaboration with the Medicial Chemistry Section, LPET, on the mechanism of action of carbocyclic nucleoside analogs. The parent analog, cyclopentenyl adenosine or neplanocin (1) proved to be an effective inhibitor of RNA methylation in human colon carcinoma cell line HT-29 (Fig. 1). Subsequent studies on the effect of neplanocin on the differentiation of human promyelocytic leukemia cell line HL-60 confirmed this activity and indicated that reduced methylation of RNA and DNA as well as the expression of the protooncogene c-myc did not lead to complete maturation of this leukemia. In contrast, the cyclopentenyl cytidine analog (2) produced rapid and complete differentiation of HL-60 cells to the granulocytic phenotype with less cytotoxicity than neplanocin. Accompanying these changes was a pronounced inhibition of CTP synthesis and subsequently, DNA synthesis, and a rapid reduction in c-myc RNA expression. Further studies of the relationship between methylation and differentiation was documented in our recent studies of 3-deazaneplanocin (c^3Nep) (3), an inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase (Fig. 1). c^3Nep has proven to be the most potent inhibitor of AdoHcy hydrolase to date and elevates intracellular levels of AdoHcy in HT-29 or HL-60 cells to an amount comparable to that of neplanocin which is also an inhibitor of the hydrolase. However, unlike neplanocin, c^3Nep is neither markedly cytotoxic, inhibitory to rRNA methylation, nor is metabolized like neplanocin to the nucleotide or S-adenosylmethionine-like metabolite. Thus, c^3Nep and Nep have allowed us to dissect the influence of inhibition of AdoHcy hydrolase on RNA



neplanocin (1)
(Nep)



cyclopentenyl cytidine (2)
(cCyd)



3-deazaneplanocin (3)
(c^3Nep)

methylation, cytotoxicity and differentiation by their diverse and specific pharmacological activities.

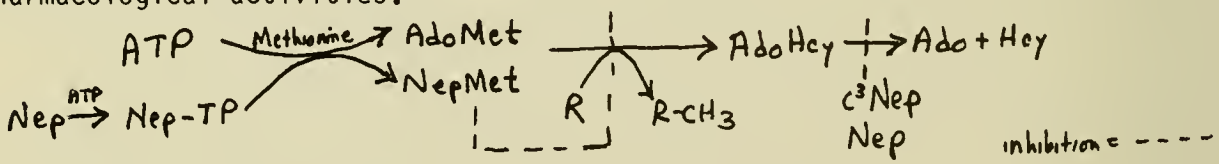


Fig. 1. Sites of action of neplanocin (Nep) and 3-deazaneplanocin (c^3Nep)

c^3Nep, although not an effective antiproliferative agent, has proven active against RNA viruses such as polio, coxsackie and vaccinia in preliminary screening.

In other studies of the antitumor activity of the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF), we ascertained that this agent was highly effective against hairy cell leukemia in phase II clinical trials. In experimental studies, we found this drug to be an effective inhibitor of the mitogenic response of T lymphocytes and to inhibit IL-2 RNA synthesis. Since the B cell

diseases, hairy cell leukemia and chronic lymphocytic leukemia have shown dependence on IL-2 and immune interferon for their growth, this finding may explain why dCF is effective against these leukemias in contrast to its ineffectiveness in acute leukemia which are not dependent on IL-2. Further studies of carbocyclic nucleoside analogs are planned. One candidate is the recently synthesized cyclopentenyl 8-azaadenosine. We previously found 8-azaadenosine to produce its cytotoxicity by its inhibition of DNA synthesis primarily via the undeaminated metabolite, 8-azaATP (Biochem. Pharmacol. 31: 3207, 1982). Deamination to 8-azaIMP and its anabolism to 8-azaGTP produced less cytotoxicity. Thus, it will be interesting to determine if the cyclopentenyl analog of 8-azaadenosine is resistant to deamination, is a more effective inhibitor of DNA synthesis and produces greater cytotoxicity in vitro. This analog may also prove to be as effective as cyclopentenyl cytidine as a differentiating agent in HL-60 cells if it blocks DNA synthesis selectively. The G_1/S phase inhibition produced by drugs of low toxicity such as cyclopentenyl cytidine appears to promote differentiation from the G_1 phase of the cell cycle in contrast to the cell cycle nonspecific effects and poor differentiating activity of drugs such as neplanocin.

Major Findings

1. The effect of the cyclopentenyl adenosine analogue, neplanocin A (Nep), on cell growth and differentiation was examined in the human promyelocytic leukemia cell line HL-60. Continuous exposure of HL-60 cells to 0.1-3.3 μM Nep resulted in a progressive reduction in cell growth which was accompanied by an increase in differentiation to cells with a myelocyte and neutrophil morphology. The latter effect was expressed as an increase in the capacity of cells to reduce nitroblue tetrazolium and reached a level of 40% of the total cell population. Preceding the phenotypic changes was the preferential inhibition of RNA and DNA methylation in comparison to inhibition of their synthesis which coincided with the formation of a metabolite of Nep with the chromatographic characteristics of S-adenosyl-L-methionine (AdoMet). In addition, c-myc mRNA expression, which is amplified in HL-60 cells, was reduced following Nep treatment. These results indicate that Nep is an effective inhibitor of RNA and DNA methylation resulting from its conversion to an analogue of AdoMet, and that these effects appear to be responsible for reduced c-myc RNA expression and the induction of incomplete myeloid differentiation in this cell line.

2. The mechanism of action of the cyclopentenyl analogue of cytidine, cCyd, was investigated in human colon carcinoma cell line HT-29. Upon exposure of cells to 10^{-6} M cCyd, cell viability was reduced to 20% of control, whereas cytocidal activity was not present after 2 hr of drug exposure. Cell lethality was partially reversible by Urd, Cyd or dCyd at 10^{-6} M cCyd, and fully reversible by these nucleosides at 2.5×10^{-7} M cCyd. The incorporation of [^{14}C]dThd and [^{3}H]Urd into DNA and RNA was inhibited by 50% by exposure for 2 hr to 2.5×10^{-7} and 1.5×10^{-6} M cCyd, respectively. Upon 24 hr of drug exposure, the IC_{50} for RNA synthesis was reduced 2.5-fold whereas DNA synthesis was almost totally inhibited. cCyd produced a rapid and preferential reduction of CTP synthesis with a half life of 1 hr at 10^{-6} M drug. The IC_{50} of cCyd for reducing CTP concentrations after 2 hr of drug exposure was 4×10^{-7}M. Concomitant with the reduction of CTP levels was the inhibition of transcription of rRNA, and to a lesser extent, tRNA,

without changes in the processing of nucleolar RNA. No changes in the size of DNA were produced following treatment with cCyd. These results indicate that cCyd is a potent and rapid inhibitor of CTP synthesis and that this effect correlates with its cytocidal activity.

3. The effects of the cyclopentenyl (cCyd) and cyclopentyl (carbodine) analogues of cytidine on differentiation, and nucleic acid and nucleotide biosynthesis were examined in human promyelocytic leukemia cell line HL-60. Continuous exposure for 5 days to 10^{-8} to 10^{-6} M cCyd or 10^{-6} to 10^{-5} M carbodine produced progressive inhibition of cell growth. During this exposure interval, pronounced differentiation to mature myeloid cells occurred wherein 95% of the cell population reduced nitroblue tetrazolium four days after exposure to 10^{-7} M cCyd or 10^{-5} carbodine. Preceding differentiation was the inhibition of DNA synthesis which reached 10% of control levels 24 hr after exposure to 10^{-7} M cCyd or 10^{-5} M carbodine, while RNA synthesis was inhibited to a lesser extent. The induction of mature myeloid cells by cCyd was preceded by the pronounced inhibition of c-myc mRNA levels which was more pronounced than the reduction in total cellular RNA synthesis. During the interval of cCyd treatment, there was a rapid and striking decrease in the level of CTP, but not of UTP, ATP or GTP, where the half-life for the reduction in CTP was 1.5 to 2 hr. Following drug removal, cells treated with cCyd showed a sustained reduction in CTP levels, whereas cells treated with carbodine showed almost complete recovery of CTP levels within 48 hr. These results indicate that the reduction in CTP levels leads to rapid inhibition of DNA synthesis and reduction in c-myc mRNA levels which precede the appearance of differentiated cells.

4. 3-Deazaneplanocin (c^3Nep), a new carbocyclic analog of adenosine, was synthesized as an inhibitor of S-adenosylhomocysteine (AdoHcy) hydrolase. The K_i of c^3Nep for a purified hamster liver preparation of AdoHcy hydrolase was 5×10^{-11} M, making this inhibitor 250-fold more potent than the previously known most potent inhibitor of this enzyme, 3-deazaaristeromycin. Inhibition was competitive with the substrate adenosine. Human promyelocytic leukemia (HL-60) cells treated with 10^{-5} M c^3Nep showed a pronounced elevation in AdoHcy which was 4-fold greater than that produced by an equimolar concentration of 3-deazaaristeromycin. This effect preceded a moderate reduction in cell growth and viability following continuous exposure for 6 days. Cellular differentiation as monitored by the reduction of nitroblue tetrazolium was not markedly affected except after 4 days exposure to 10^{-5} M c^3Nep where 60% of the viable cells were positive. These results indicate that c^3Nep may have therapeutic potential as an anticancer or antiviral drug.

5. 3-Deazaneplanocin (c^3Nep) was tested as an antiprolifeerative agent against human colon carcinoma cell line HT-29. c^3Nep produced a 16 and 34% reduction in cell viability after 24 h of drug exposure at 10 and 100 μM concentration, respectively. Neplanocin at identical concentrations decreased viability by 31 and 87%. c^3Nep inhibited AdoHcy hydrolase in vivo as assessed by elevation in AdoHcy levels, and was found to be as effective as neplanocin as an inhibitor of AdoHcy synthesis. However, unlike neplanocin, c^3Nep neither markedly inhibited RNA methylation nor was metabolized to an S-adenosylmethionine-like metabolite. This result suggests that inhibition

of AdoHcy hydrolase, while a good antiviral target, is not effective in producing a tumoricidal response.

6. 2'-Deoxycoformycin (dCF) was tested for its antimitogenic effects against mouse lymphocytes and its ability to inhibit IL-2 RNA synthesis in vitro. dCF directly inhibited lymphoblastogenesis only when present at the beginning of mitogen treatment, a time period where production of IL-2 mRNA occurred. These temporal similarities suggest that dCF may be producing its immunomodulatory effects via suppression of IL-2 synthesis. This hypothesis was tested by hybridization studies using [^{32}P]DNA probe for IL-2 to measure IL-2 RNA synthesis. A strong hybridization signal was obtained as early as 3 h after treatment with mitogen and TPA. Synthesis of IL-2 mRNA was inhibited by dCF alone, as well as by antimitogenic concentrations of adenosine or deoxyadenosine. This effect was further accentuated when adenosine was coadministered with homocysteine, indicating that dCF can produce immunosuppressive effects either by itself or via subsequent metabolic imbalances as a result of inhibition of adenosine deaminase and lymphocytolysis. Results with mycophenolic acid indicate the early reduction in the IL-2 mRNA level is not caused by a general inhibition of RNA synthesis. Our results may have implications in the progression of lymphoid malignancies such as mycosis fungoides, chronic lymphocytic leukemia, and hairy cell leukemia which are highly responsive to dCF therapy. The reason for their sensitivity may reside in their dependence, in part, on the lymphokines IL-2 and immune interferon for continued growth. The inhibitory effect of dCF on IL-2 mRNA suggests that an interleukin-2-dependent growth component in these diseases is likely.

7. Eight patients with hairy-cell leukemia (HCL) complicated by pancytopenia, were treated with low dose regimens of the adenosine deaminase (ADA) inhibitor dCF. All patients had significant hematological and clinical improvement. One patient who had been splenectomized and 5 patients with mild to moderate splenomegaly achieved normal blood counts within two months, which have been maintained for up to 18 months. Complete remissions occurred in two patients and four patients had 50-95% marrow clearance of hairy-cells. The initial dCF treatments produced a 1-3 gm/dl fall in the hemoglobin levels and one patient had a temporary reduction in granulocyte and platelet counts. Five patients had nausea/vomiting, and/or lethargy following dCF, but there was no correlation between the plasma levels of deoxyadenosine and adenosine and the incidence or severity of these side effects. An increased incidence of infection and drug hypersensitivity may reflect the effects of dCF on immune system. Low dose dCF is a highly effective agent in HCL.

Publications

1. Cohen, M.B. and Glazer, R.I.: Inhibition of interleukin-2 messenger RNA in mouse lymphocytes by 2'-deoxycoformycin and adenosine metabolites. Ann. N.Y. Acad. Sci. 451: 180-187, 1985.

2. Johnston, J.B., Israels, L.G. and Glazer, R.I. The treatment of hairy cell leukemia with 2'-deoxycoformycin. Ann. N.Y. Acad. Sci. 451: 319-320, 1985.

3. Glazer, R.I., Knode, M.C., Lim, M.-I. and Marquez, V.E.: Cyclopentenyl cytidine analogue: an inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. Biochem. Pharmacol. 34: 2535-2539, 1985.

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5. Glazer, R.I. Hartman, K.D., Knode, M.C., Richard, M.M., Chiang, P.K., Tseng, C.K.H. and Marquez, V.E.: 3-Deazaneplanocin: a new and potent inhibitor of S-adenosylhomocysteine hydrolase and its effects in human promyelocytic leukemia cell line HL-60. Biochem. Biophys. Res. Commun. 135: 688-694, 1986

6. Glazer, R.I., Cohen, M.B., Hartman, K.D., Knode, M.C., Lim, M.-I. and Marquez, V.E.: Induction of differentiation in human promyelocytic leukemia cell line HL-60 by the CTP synthetase inhibitor, cyclopentenyl cytidine analogue. Biochem. Pharmacol., in press, 1986.

7. Johnston, J.B., Glazer, R.I., Pugh, L. and Israels, L.G.: The effectiveness of 2'-deoxycoformycin in the treatment of hairy cell leukemia. Brit. J. Haematol., in press, 1986.

8. Glazer, R.I., Knode, M.C., Tseng, C.K.H., Haines, D.R. and Marquez, V.E.: 3-Deazaneplanocin: a new inhibitor of S-adenosylhomocysteine hydrolase and its effects in human colon carcinoma cells. Biochem. Pharmacol., in press, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07156-03 LBC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Differentiation of Human Leukemia Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Theodore R. Breitman	Chemist	LBC, NCI
Others:	Masue Imaizumi	Visiting Fellow	LBC, NCI
	Jiro Uozumi	Visiting Fellow	LBC, NCI

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.3	2.3	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recently, there has been interest in the possibility that "differentiation-inducers" may have utility in the treatment of some malignancies. This concept is predicated on the belief that some malignancies are a result of a block in differentiation which if relieved would result in a more differentiated and therefore more benign condition. As a concept for therapy this approach holds the further promise that induction of differentiation could not only relieve the tumor burden but also increase the number of functional cells, that at least for some malignancies, an absence of is a major complication. HL60 has been a useful model system in the search for substances that are active as inducers of differentiation. HL60 is induced to differentiate to granulocyte-like cells by incubation with retinoic acid (RA), DMF, and DMSO or into monocyte/macrophage-like cells by incubation with 1,25-dihydroxyvitamin D_3 and TPA. Of the many compounds that induce differentiation of HL60, RA has probably the most promise of being of use in the clinic. To the extent that it is possible, results *in vitro* should suggest treatments *in vivo*. To this end we have studied the differentiation effect of RA in nude mice carrying a transplantable HL60 tumor. There was no increase in life-span of treated animals and no evidence that tumor cells were induced to differentiate. These results were even more surprising because the tumor cells, established in culture and designated HL60/MRI, were found to be 100-fold more sensitive to RA *in vitro* than the parent HL60. In addition, RA induces HL60/MRI to differentiate to monocytoid cells. RA binds to serum albumin in a specific manner with an equilibrium constant of association of $10^6 M^{-1}$. Thus, even at pharmacological concentrations of 1 μM all of the circulating RA is bound to albumin. This binding may be a major factor for the absence of antitumor activity of RA *in vivo* as serum and/or serum albumin inhibit RA induced differentiation of HL60 *in vitro*.

Objectives

This project seeks an understanding of the process of terminal differentiation of myeloid cells. It is now generally agreed that some leukemias, as well as other malignancies, are diseases resulting from a block in terminal differentiation. This view suggests that viable treatment may be possible with agents that induce differentiation. To aid in this search, studies are conducted to: a) better understand the mechanism(s) of terminal differentiation; b) study the metabolism of known inducers of differentiation, e.g., RA, to aid in the development of more potent inducers; c) screen known and newly synthesized compounds for their differentiation inducing activity; d) employ an animal model system (transplantable HL60 carried in athymic nude mice) to determine if inducers of differentiation *in vitro* are active by a similar mechanism *in vivo*; e) establish as transplantable tumors in athymic nude mice other human myeloid leukemia cell lines to be used for testing protocols developed for treating the HL60 transplantable tumor line.

Methods Employed

The principal methods employed involve measurement of differentiation of human leukemia cell lines in cell culture. Most studies are conducted with the HL60 promyelocytic cell line. Differentiation is assessed primarily by morphology and the ability of cells to reduce nitroblue tetrazolium to a formazan after incubation of HL60 cells for 4-5 days in the presence of an inducer.

Major Findings

1. We confirmed the finding of Bogden and Cobb that the transplantable HL60 tumor, designated HL60/MRI, grows in athymic nude mice. However, *in vivo* there was neither RA-induced differentiation nor an increase of lifespan of treated mice.

2. As part of studies to understand why HL60/MRI did not respond to RA *in vivo* the tumor cells were established as a cell line *in vitro*. These cells differentiate to monocytes when cultured in the presence of RA. RA-induced HL60/MRI exhibit markers and functions of monocytes such as: monocyte-specific OKM5 antigen, positive reaction for non-specific esterase, 12-O-tetradecanoylphorbol-13-acetate (TPA) stimulated superoxide production, and adhesiveness. While TPA also induces HL60/MRI to monocytoid cells, as it does HL60, dimethylsulfoxide and hexamethylene bisacetamide induces HL60/MRI to granulocytes and not to monocytes. In addition to this unique monocytic differentiation by RA, HL60/MRI compared to HL60, differentiates in response to RA at a rate approximately two-fold greater and at RA concentrations approximately 100-fold lower. The karyotype of HL60/MRI is characterized with hyperploid chromosomes and with a 16q+ that are not observed in HL60.

3. A new method has been developed to measure superoxide production by maturing HL60 cells. The method uses the incubation conditions of the "standard NBT test" but instead of measuring the percentage of cells that have NBT-formazan deposits the amount of formazan produced is measured spectrophotometrically after extraction of the formazan with alkaline dimethylformaide.

4. The binding of RA to serum albumin has been measured in a quantitative manner for the first time. Scatchard graphical analysis indicates that there is one class of binding sites with a Ka of approximately 1 μM^{-1} and with 1 to 3 binding sites per molecule of albumin. There is a second class of binding sites with a Ka of 6 nM^{-1} and with 20 sites per molecule of albumin. Because of this very low affinity it is unlikely that this second class of binding sites has physiological significance. However, based on the binding analysis and the concentration of serum albumin (60 mM) it is likely that essentially all of the circulating RA (30 nM) is bound to albumin. In addition it is calculated that most of a pharmacological concentration of RA (1 μM) is also bound to albumin. These kinetic analyses give a physico-chemical basis for the observations that serum and/or serum albumin inhibit RA-induced differentiation of HL60 *in vitro*. In addition, this binding may contribute to the lack of an effect of RA *in vivo* against the transplantable HL60 tumor (see 1 above).

5. The binding of gammainterferon to HL60 cells has been measured. To explain a previous observation that combinations of RA and gamma-interferon are synergistic in inducing differentiation of HL60 we explored the possibility that RA induced an increase in surface interferon receptors. No increase was observed.

Proposed Course

1. The specific binding of RA to serum albumin has both theoretical as well as practical implications. It will be of interest to explore if the RA binding site(s) are the same site(s) to which fatty acids bind. Competition experiments between fatty acids and RA will clarify this point. Results from these experiments should suggest agents that can displace RA from albumin and lead to an increase in the effective concentration of RA, both *in vitro* and *in vivo*.

2. The possibility will be explored that the lymphokine DIA binds to the gamma-interferon receptors of HL60. If true this can explain the very similar actions of these two lymphokines.

3. The unique RA-induced differentiation of HL60/MRI to monocytoid cells is the basis for an investigation of the expression of various oncogenes. It has been demonstrated that during TPA-induced differentiation of HL60 to macrophages there is a very early marked increase in the expression of c-*fos* followed by a later increase in c-*fms*. Induction of HL60 to granulocytes by dimethylsulfoxide and hexamethylenebisacetamide is not accompanied by changes in these oncogenes. These results have been interpreted to indicate that these changes in oncogene expression are monocyte specific. We plan to look at the expression of various oncogenes including *fms* and *fos* during the RA-induced differentiation of HL60/MRI to monocytes using HL60 as a control. Results obtained from this study may throw new light on oncogene expression and may lead to an explanation as to why HL60/MRI does not respond *in vivo* to RA treatment.

Publications

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3. Breitman, T.R., Hemmi, H. and Imaizumi, M. Induction by physiological agents of differentiation of the human leukemia cell line HL60 to cells with functional characteristics. In Serrero, G., Hayashi, J., Sato, G. (Eds.) Symposium on Cellular Endocrinology. From Embryo to Cells: Hormonal Control of Differentiation. New York, A. Liss, 1986, in press.

ANNUAL REPORT OF THE LABORATORY OF EXPERIMENTAL THERAPEUTICS AND METABOLISM

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Laboratory of Experimental Therapeutics and Metabolism (LETM) personnel conduct experiments and generate basic information that contributes to the improvement of cancer treatment. This interdisciplinary research program in tumor biology, pathology, biochemistry, pharmacology, and toxicology has overall aims that include: 1) utilization of the biochemical and pathobiological characteristics of hepatic and extrahepatic tumor cell populations to define the histogenesis of specific cancers; 2) establishment of specific animal and human tumor cell lines to study new and existing anticancer drugs for their mechanism of toxicity; 3) investigation of biochemical and molecular mechanisms of drug metabolism and drug toxicity; 4) elucidation of the metabolic and physiological factors that may underlie target organ and target cell specific toxicity and prevention of toxicity by cellular defense mechanisms; and 5) the continuation of the development of suitable cellular and animal models to elucidate mechanisms of toxicity of anticancer drugs and chemical modulators of anticancer drug effects.

While maintaining these general aims, there has been a substantial consolidation and reprioritization of specific research projects during this past year. This was brought about by 1) a substantial reduction in LETM resources and 2) a need for additional basic research to complement the major thrust of the DTP to discover tumor-specific antitumor agents. Much of this needed expertise existed in the LETM. A minor reorganization and refocusing of objectives allowed the formation of a smaller, more cohesive, LETM that plays an essential complementary role in the larger DTP project but yet retains the individual research interests of its senior investigators. All of the 15 projects listed in the Annual Report for 1985 were either altered or deleted and four new projects formulated that more clearly reflect the long-range interests of the LETM.

The LETM is presently organized into two Sections.

Pharmacology and Toxicology Section.

As part of the LETM, the overall goal of the Pharmacology and Toxicology Section is to provide basic information pertaining to the biochemistry, pharmacology, and toxicology of chemicals that are useful in our understanding and improvement of cancer therapy. Personnel in the Pharmacology and Toxicology Section utilize an interdisciplinary and collaborative approach to the project areas summerized below.

a) Human Lung Tumor Project

This project is an important component of the DTP's effort to discover

drugs specific for the treatment of human lung cancer. Classification of lung tumors with regard to their metabolic potential is particularly important for developing a systematic approach for the identification of active antilung tumor agents. We have, therefore, initiated the clinical lung cancer research project in order to develop a relevant and feasible plan for classification of human lung tumors, as well as for the identification of effective lung cancer treatment modalities. This project interfaces the clinical aspects of human lung cancer with basic science and applied science technologies which are currently available in an effort to provide a comprehensive program for characterization of lung tumors based on their morphological, ultrastructural, biochemical, metabolic, and molecular genetic characteristics as well as to establish in vitro and in vivo techniques for screening potential antitumor drugs against primary human lung tumors. The intital results from our laboratories document the feasibility and relevance of this project by demonstrating: (1) considerable variation in prostanoid as well as 4-ipomeanol metabolism among individual fresh human lung tumor specimens, (2) the ability to establish and propagate primary lung tumor cell cultures which will provide material for in vitro drug screening, establishing long term tumor cell lines, and for comparative biochemical studies, and (3) the ability to propagate human lung tumors in the lungs of nude mice by a novel intrabronchial implantation method which will allow for improved in vivo propagation and drug screening studies. It is anticipated that this project will provide a paradigm for individualized lung cancer characterization and treatment.

b) Prostaglandins and Related Eicosanoids in Human Cancer

As mentioned above, elucidation of important metabolic pathways in specific tumor types could aid in the development of tumor-specific agents. With the availability of well classified human lung tumors, a project to study eicosanoid metabolism in these cell lines was initiated to: 1) discover new targets for drug manipulation, and 2) to determine if eicosanoid levels could be used as a diagnosis for specific tumor types.

The role of the prostaglandins and related eicosanoids in several pathophysiologic aspects of human malignant disease has been the subject of a number of intense investigations. This family of compounds has been implicated in the modulation of cell proliferation, tumor promotion, immunoregulation and metastatic tumor dissemination. The development of methods in the LETM for the simultaneous qualitative and quantitative analysis of the five principal prostaglandins and their metabolites provides the analytical basis for determination of the role of this group of highly active compounds in the pathophysiology of human lung cancer and other human malignancies. Our observation that prostaglandin E_2 is the most prominent prostanoid biosynthesized by two human lung tumor lines (NCI-H322 and NCI-H358) in response to exogenous stimuli suggest that prostanoid biosynthesis may be a unique biochemical characteristic of a group or subclass of human primary lung carcinomas. Studies relating prostaglandin biosynthetic capabilities of human primary lung carcinomas in vivo and in vitro have been initiated. The in vitro studies of prostanoid production employ fresh human lung carcinoma isolates and the primary and early passage cultured cells derived from human lung carcinoma isolates.

The assessment of in vivo production of prostanoids, particularly prostaglandin E_2, will employ newly diagnosed, human lung cancer patients whose tumors have been classified by histopathologic criteria. Prostaglandin E_2 production in vivo in this patient population will be determined via the urinary excretion rate of the major urinary metabolite of PGE_2 (7 α-hydroxy-5,11-diketo-tetranorprostane-1,16-dioic acid; PGE-M) via high resulution capillary gas chromatography-negative ion chemical ionization mass spectrometry.

c) Development of Tumor-Specific Chemotherapy

Basic information in areas of biochemistry, pharmacology and toxicology can contribute to the improvement of cancer treatment. This project investigates the biochemical and molecular mechanisms of drug metabolism and drug toxicity, elucidation of the metabolic and physiological factors that may underlie target organ and target cell specific toxicity, and prevention of toxicity by cellular defense mechanisms. Information of this type, obtained for normal tissues and specific tumor types, can be utilized to provide 1) a more rational approach to increase the therapeutic effectiveness of anticancer agents while simultaneously reducing their potential for untoward toxic effects and 2) a basis for target cell-oriented cancer therapy in drug development. We have demonstrated that a model compound 2-bromohydroquinone can be cooxidized in vitro by renal prostaglandin synthase to covalently bound material and glutathione adducts. This cooxidation process produced a concentration dependent alteration of prostaglandin synthesis. In a parallel study, prostaglandin synthese activity was determined in selected human lung carcinoma cell lines and was found to have varying prostaglandin synthase activity listed in ascending order - NCI-H69, NCI-H128, NCI-H322, NCI-H358 and NCI-H460. These human lung carcinoma cell lines were employed as models to explore the ability of the different human lung cancers to activate xenobiotics, especially antitumor agents directed against the prostaglandin synthase system in the hope of achieving a more selective therapeutic effectiveness. The concept of searching for certain enzymes in relation to tumor development has been expanded to examine another enzyme, γ-glutamyltranspeptidase. γ-glutamyltranspeptidase is widely used as a marker in preneoplastic lesions in the liver during chemical carcinogenesis. We have demonstrated that 2-bromohydroquinone gives rise to a mixture of isomeric mono- and disubstituted glutathione conjugates, the latter being a potent nephrotoxin. In addition, we have demonstrated that selective transport of 2-bromohydroquinone glutathione conjugates into kidney may be mediated by γ-glutamyltranspeptidase and that the more extensive renal uptake of the disubstituted glutathione may be partially responsible for its enhanced nephrotoxicity. It is thus conceivable that these studies on the modulation and mechanism(s) of glutathione conjugate mediated toxicity might enable a more rational application of such compounds as potential anticancer agents.

Pathology and Ultrastructrual Oncology Section

This Section's research focuses on mechanisms of development and therapy of lung cancer. Studies on the pathogenesis of chemically induced lung cancer in rodents as well as reports on human lung cancers have provided

ample evidence that the histopathologic typing of lung cancers and their preneoplastic lesions is insufficient for research in that they lack information on cell type and degree of differentiation of subcellular organelles. Projects of this Section and collaborative efforts with the Pharmacology and Toxicology Section, LETM, as well as other laboratories are therefore based on the concept that response of cells to chemicals including drugs are dictated by specific biochemical reactions of cells in a manner which can be dependent on cell types and differentiation. This approach necessitates the application of electron microscopy for diagnosis of cell type and degree of differentiation at the organelle level to all experiments. Moreover, studies on the effects of chemicals and drugs are generally addressed with the same methodology to provide initial information on target cell type(s) and/or organelles that could then be utilized for the design of more focused experiments on the underlying biochemical mechanisms.

Our major emphasis during this report period has been on the characterization and establishment of human lung cancer cell lines (HLCCL) for research on cell type specific chemical/drug interactions. The experience generated through such studies has also enabled us to initiate and design a number of experiments primarily conducted by investigators of the Pharmacology and Toxicology Section, LETM. We have demonstrated that certain human lung cancer cell lines, both of the small cell lung cancer (SCLC) and the non-small cell lung cancer (NSCLC) types, retain the ultrastructural and biochemical characteristics of specific cell types present in the lung in vivo. These cell lines have been characterized extensively and have served as model systems to study many diverse but clinically relevant aspects of human lung cancer cell biology. In addition to the collaborative studies with the Pharmacology and Toxicology Section discussed above, we have used these cell lines in collaborative studies on the mechanisms of action of several chemotherapeutic agents including arabinosyl-5-azacytosine, merbarone, 2',3'-dideoxycytidine, and BCNU. A well characterized rat alveolar type II cell system has also been developed to study the effects of toxins, carcinogens and anticancer drugs on normal lung cells.

A new study investigates the process of carcinogenesis to reveal possible targets for chemical intervention to reverse the transformed state. Carcinogenesis and cancer chemotherapy may be related from a cellular standpoint in the search for tumor-specific agents. Accordingly, a thorough understanding of the biology of normal cell types and their alterations during cellular transformation may therefore allow for rational approaches to both cancer prevention as well as effective cancer chemotherapy. Liver cancer cell biology studies which involve chemical transformation of normal rat liver epithelial cells demonstrated for the first time that there exists a clonal relationship between the two most prominent types of liver cancer, namely hepatocellular carcinomas and cholangiocarcinomas. These studies may lead to the elucidation of the cell types responsible for liver cancer and to more effective therapy. Moreover, the in vitro hepatocarcinogenesis studies revealed that malignant transformation of rat liver epithelial cells results in the loss of proliferative control exerted by two endogenous polypeptide growth factors, hepatic proliferation inhibitor (HPI) and transforming growth factor Beta (TGF).

These transformed cells in conjunction with normal hepatic cells may be useful in elucidating the mechanism(s) of action of HPI and TGF.

The preceding summary outlines the objectives of the Laboratory of Experimental Therapeutics and Metabolism and highlights some of the research carried out within the Laboratory during the past year. The individual Project Reports, which follow, describes this research in greater detail.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07119-07 LETM

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies on the Biochemical Toxicology of Oncolytic Platinum Compounds

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C. L. Litterst Pharmacologist LETM, NCI

COOPERATING UNITS *(if any)*

LPET (Dr. J. Uozumi)

LAB/BRANCH
Laboratory of Experimental Therapeutics and Metabolism

SECTION
Pharmacology and Toxicology Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Although the toxic effects of cisplatinum on kidney have been appreciated for some time, the renal handling of cisplatinum and the mechanism by which the renal toxicity occurs are still incompletely understood. These mechanisms could be more easily defined if the molecular sites of interaction of cisplatin were recognized. This project is designed to define how the kidney handles cisplatin under normal conditions and after various pretreatments or other experimental conditions. Inherent in this study is an attempt to localize the sites of interaction of cisplatin and its intracellular binding sites. This section reports the sex and tissue specificity of platinum binding to DNA, the effect of cisplatinum on renal ATPase function in vivo and in vitro and the blockage of cisplatin-induced renal toxicity by sodium thiosulfate.

PHS 6040 (Rev. 1/84) GPO 914-918

Studies on the mechanism of action of cisplatin:

Much work has been done to document the DNA binding of cisPt and to establish this as the probable mechanism of the antitumor effect. Similarly, much effort has been invested in documenting the various toxicities produced by cisPt, although the mechanisms of these effects have not yet been satisfactorily established. There is an obvious interest in attempting to establish whether there is any relation between DNA binding and renal toxicity. We have therefore investigated the relation between DNA binding as measured by an ELISA technique which is specific for the N-6 DNA adduct with cisPt, and various parameters of platinum toxicity and pharmacokinetics. We have documented an apparent sex-related specificity in the uptake of cisPt into renal nuclei between male and female rats. Although the total renal concentration of Pt is greater in female rats, the DNA binding in females is less than in males. This finding is consistent with clinical response of ovarian and testicular cancer patients and with the differential response observed between older (post-menopausal) and younger (pre-menopausal) ovarian cancer patients. The implication is that there is a hormonal influence on Pt binding or transport which is responsible for the lesser amount of binding in females. A related organ-specificity also was noted, with testicles accumulating substantially greater amounts of DNA adducts that did ovaries. This is again consistent with the greater clinical response seen in testicular cancer patients than in ovarian patients. Finally, in both kideys and gonads of males and females, DNA binding but not whole tissue platinum content, appears to be dose dependent, with a dramatically elevated increase in binding at high cisplatin doses. This suggests, among other things, that the transport of platinum into the nucleus, or the cytosolic binding of platinum, may be saturable. These early results are currently in manuscript form, and experiments are continuing to investigate the mechanism behind these findings.

The effect of cisplatin on content and function of renal ATPase was investigated as a possible explanation for the renal toxicity of the drug. When purified ATPase or when ATPase from tissue homogenates were incubated in vitro with cisplatin decreases in ATPase activity were observed. However, either very high concentrations of cisplatin (400-600 uM) or very long times of incubation (2-3 hrs.) were required before any changes were noted. In vivo, cisplatin produced a decrease in ATPase that occurred several days after drug administration and 1-2 days after significant elevations of BUN occurred. Renal platinum concentrations were below the concentrations required to produce significant decreases in activity when tested in vitro. It was concluded that cisplatin probably does not interact with ATPase as a primary site of toxicity, but that cisplatin does have the ability to decrease the activity of this enzyme as a secondary effect.

Sodium thiosulfate (STS) was shown to completely block the cisplatin induced rise in BUN and to prevent cisplatin-induced deaths. The mechanism of this interaction was investigated by examining the subcellular distribution of platinum in rats receiving cisplatin and in rats receiving cisplatin plus STS. Renal platinum concentrations in STS pretreated rats were less in nuclei, mitochondria, and microsomes during the first 8 hours after cisplatin administration. Similar decreases in platinum content were not consistently observed in liver. The differences in distribution were statistically signif-

icant but were of a relatively small magnitude, so the biological significance is questionable. Because of this small changes in distribution, it is probably not realistic to ascribe the dramatic blockage of renal toxicity entirely to changes in subcellular distribution of platinum.

Depletion of tissue levels of glutathione by pretreatment with either Buthionine sulfoximine (BSO) or diethylmaleate (DEM) resulted in a dramatic increase in cisplatin lethality and renal toxicity. There were relatively minor increases in tissue concentration of platinum. After pretreatment with DEM or BSO platinum concentrations were increased in mitochondria, cytosol, and to a lesser extent in microsomes at nearly all times between 2 minutes and 24 hr after a single dose of cisplatin. Pretreatment of animals with reduced glutathione (GSH) resulted in decreased lethality and decreased renal toxicity of cisplatin. Tissue concentrations of Pt also were decreased. Concentration of Pt in subcellular organelles showed more variability. Cytosol concentrations were increased and microsomal concentrations were decreased throughout the first 24 hr after cisplatin injection.

Increasing the amount of a cadmium-binding protein which binds platinum produced a decrease in the toxicity of high doses of cisplatin but produced no related changes in tissue platinum concentrations. Platinum concentrations in subcellular fractions were increased in mitochondria and cytosol at 8 and 24 hr but not at 2 minutes after cisplatinum treatment. It thus appears as of both glutathione and cadmium binding protein may act as non-specific binding sites for cisplatin.

Pharmacokinetics and toxicity of experimental drugs:

Tetraplatin is a new analog of cisplatin that has shown activity in animal tumor systems which are resistent to cisplatin. We have conducted extensive preclinical toxicity studies of this compound in comparison with cisplatin and CHIP, another cisplatin analog with chemical similarity to tetraplatin. We evaluated the effect of 4 equimolar doses of the drugs on renal histology, urinary and plasma enzymes, and renal slice function over a 15 day time course. It appears from early inspection of the data, as if tetraplatin is less nephrotoxic, but that the toxicity is not dramatically less. In addition, the histopathologic distribution of the lesion is generally throughout the cortex, rather than being restricted to the corticomedullary junction, as is the case with cisplatin. We are continuing to evaluate our data so that we we will be able to draw more definite conclusions regarding the relative toxicity of this analog.

This project was previously reported under Project #Z01 CM 07119-06 LPET.

Publications:

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2. Litterst, C.L.: Cisplatinum: A review, with special reference to cellular and molecular interactions. Agents Actions 15: 520-525, 1985.

3. Litterst, C.L.: Toxicology of the Antineoplastic Agents, with Special Reference to Reproductive Toxicology, Carcinogenesis, and Teratogenesis. In Haley, T. and Berndt, W., (Eds.): Handbook of Toxicology, New York, Hemisphere Publishing Corp., in press.

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6. Farris, F.F., King, F.G., Dedrick, R.L. and Litterst, C.L.: Physiological model for the pharmacokinetics of cis-dichlorodiammineplatinum (II) (DDP) in the tumored rat. J. Pharmacokin. Biopharm., in press.

7. Uozumi, J. and Litterst, C.L.: Effect of cisplatin on renal ATPase activity in vivo and in vitro. Cancer Chemotherapy & Pharmacology, in press.

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9. Litterst, C.L., Smith, M., Smith, J., Uozumi, J. and Copley, M.: Sensitivity of urinary enzymes as indicators of renal toxicity of the anticancer drug cisplatinum. In Bianchi, C. and Blaufox, D.M. (Eds.): Newer Diagnostic Methods in Nephrology and Urology. Basel, Switzerland, Karger Publishing Co., in press.

NOTE: This project was previously reported under the Laboratory of Pharmacology and Experimental Therapeutics, DTP

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07162-03 LETM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Prostaglandins and Related Eicosanoids in Human Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	W. C. Hubbard	Cancer Expert	LETM, NCI
Others:	T. L. McLemore	Senior Investigator	LETM, NCI
	C. C. Litterst	Pharmacologist	LETM, NCI
	M. R. Boyd	Associate Director	DTP, DCT

COOPERATING UNITS *(if any)*

Johns Hopkins University School of Medicine (Dr. Liu)
Program Resources, Inc. (Dr. Alley)

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolsim

SECTION

Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Cell culture methods were modified for the NCI-H322 and NCI-H358 cells to obtain a system for kinetic studies of prostanoid synthesis from arachidonic acid free of perturbations in temperature and gas equilibrium. Unidispersed suspensions of trypsinized cells were attached to Cytodex-1 microcarriers at a ratio of 35-40 cells per microcarrier. After culturing at 37° in 5% CO_2: 95% water-saturated air atmosphere for 2-3 days the microcarriers were almost completely covered. The microcarrier attached NCI-H322 and NCI-H358 cells were prepared for experimentation via two washes with Hank's balanced salt solution containing Ca^{++} and Mg^{++} (HBSS+) followed by suspension in HBSS+ containing glucose (2mg/ml). Quantitative and qualitative analysis by high resolution capillary gas chromatography negative ion chemical ionization mass spectrometry indicate that these cell lines derived from human bronchioloalveolar carcinomas synthesize PGE_2, PGD_2 and $PGF_{2\alpha}$ in response to stimulation by exogenous arachidonic acid and by the ionophore A23187. The response of the NCI-H322 and NCI-H358 cells to these stimuli are rapid with prostanoid synthesis being complete within four minutes. PGE_2 is the most abundant arachidonate metabolite synthesized in there two human lung carcinoma cells. In addition to studies of arachidonate metabolism in the NCI-H322 and NCI-H358 cells, high resolution capillary gas chromatography mass spectrometry has also been employed to assess prostanoid production in human lung carcinoma tissue, renal cell carcinoma tissue, in tumor tissue from nude mice bearing human lung carcinomas and from normal tissues in which the tumors were resident. Our studies suggest that prostanoid synthesis may be a characteristic unique to a group or subclass of human malignancies and may be relevant diagnostically and in the classification of human tissues.

METHODS DEVELOPMENT

Our initial studies of arachidonate metabolism in four cell lines derived from human lung carcinomas indicate that NCI-H322 and NCI-H358 cells selectively synthesize prostaglandins. These two cell lines proliferate as adherent cells. In the absence of special facilities, it is difficult to acquire samples from monolayers essential for studies of the kinetics of prostanoid biosynthesis in these cell lines without adverse perturbations in temperature and gas equilibrium. We have developed methods in which the NCI-H322 and NCI-H358 cells are attached to Cytodex-1 (Pharmacia) microcarriers and employed as a system for obtaining samples essential for studies of the kinetics of prostanoid biosynthesis. Typically, monodispersed NCI-H322 and NCI-H358 cells are added to a flask at a carrier/cell ratio of 35-40:1 and stirred for two hours at 15-20 rpm at 37° in a volume of 100ml of complete RPMI 1640 media. After the addition of complete RPMI 1640 media to a final volume of 250 ml, the suspension culture is maintained at 37°C in a 5% CO_2 - 95% water-saturated air atmosphere for 2-3 days. At this time, the microcarriers are almost completely covered as determined by microscopic examination. The microcarrier-attached cells are prepared for experimentation via removal of the complete RPMI-1640 medium, washing twice with Hank's balanced salt solution containing Ca++ and Mg++ (HBSS+) and finally suspension in HBSS+ (with glucose, 2mg/ml) at a density of $1\text{-}5x10^6$ cells/ml. The number of microcarrier attached cells in each experiment is determined by cell counting (Coulter counter) subsequent to removal of cells from the microcarriers via trypsinization.

The NCI-H322 and NCI-H358 cell lines (monodispersed cells following trypsinization, monolayers and microcarrier-attached) were also employed in the development of methods for qualitative and quantitative analysis of prostanoids via high resolution capillary gas chromatography-mass spectrometry employing a Finnigan MAT 4610B GC/MS/DS. Samples of incubation media prepared for analysis by this system were evaluated for sample purity and in the optimization of conditions for vapor phase and mass spectral analysis. We have shown that prostanoids present in extracts from NCI-H322 and NCI-H358 cells and in certain unextracted samples are readily amenable to simultaneous qualitative and quantitative analysis by high resolution capillary gas chromatography-mass spectrometry. Our procedures are described below.

After the addition of 0.8 to 2.0 ng of tetradeuterated ($^2H_4$) analogs of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$, each sample is sequentially subjected to conditions for oximation, esterification and silylation of functional groups of the prostanoids. After derivatization, the prostanoids are extracted with hexane and dissolved in 40µl of dodecane in preparation for analysis. Typically, each injection of these samples represents 1-2.5% of the sample. We have observed that standard curves employing standards of $PGF_{2\alpha}$, PGD_2, PGE_2, thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ are linear over a range of $(^2H_0)/^2H_4$ ratios of 0.00 (blank) to 1.200. The detection limits for each injection is 0.1 to 0.2 picograms of prostanoids present in each injection when negative ion chemical ionization mass spectrometric analysis is employed.

In addition to the development of methods for analysis of the primary prostaglandins, we have also developed methods for the simultaneous qualitative and quantitative analysis of the 15-keto-13,14,-dihydro-metabolites of PGE_2, PGD_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ by high resolution capillary gas chromatography-mass spectrometry. In the absence of commercially available deuterated analogs of these compounds, deuterated analogs of each of the 15-keto-13,14-dihydro metabolites was prepared via synthesis of the trideutero ($^2H_3$) analog

of the methyloxime derivatives. The mono- and di-$^2H_3$ analogs were added to samples in standard quantities subsequent to complete derivatization. We observed $^2H_0/^2H_3$ ratios of $\leq$ 0.0002 (less than 0.2 parts/thousand) and detection limits of less than 0.2 picograms per injected sample. Standard curves were found to be linear from $^2H_0/^2H_3$ ratios of 0.00 (blank) to 1.40.

MAJOR FINDINGS

The profile of prostanoids synthesized by the NCI-H322 and NCI-H358 cells from exogenous arachidonic acid (1-50μM) by monodispersed cells (immediately subsequent to trypsinization), by cells in monolayers in culture flasks and by cells attached to microcarriers was qualitatively similar. Three prostaglandins identified by high resolution capillary gas chromatography - mass spectrometry as PGE_2, PGD_2 and $PGF_{2\alpha}$ were synthesized by the NCI-H322 and NCI-H358 cells. PGE_2 was the most abundant of the prostanoids synthesized from exogenous and endogeneous arachidonic acid in all three of the cell preparations employed. Quantitative determinations of prostanoid biosynthesis from endogenous arachidonic acid in the presence of 5uM ionophore A23187 in monodispersed, trypsinized cells indicated that NCI-H322 and NCI-H358 cells immediately subsequent to trypsinization do not synthesize detectable quantities of prostaglandins. Conversely, ionophore A23187-stimulated cells attached to microcarriers readily synthesized prostaglandins from endogenous arachidonic acid. Subsequent to our findings summarized above, we employed microcarrier-attached suspensious of NCI-H322 and NCI-H358 cells for kinetic studies of prostanoid formation in these cells. Stimuli employed were as follows: 1) none (including vehicle controls); 2) exogenous arachidonic acid in final concentrations of 2,10 and 50μM (dissolved in absolute ethanol; final vehicle concentration $\leq$ 2.5%); and 3) calcium ionophore A23187 in final concentrations of 1,5 and 10μM (dissolved in absolute ethanol: dimethylsulfoxide; 50:50; final vehicle concentration = 2.5%). In the absence of a stimulus, PGE_2 levels synthesized by the cells were less than 0.4 picomoles/10^6 cells. PGD_2 and $PGF_{2\alpha}$ were not detectable. The response of the NCI-H322 and NCI-H358 cells to the ionophore A23187 and to exogenous arachidonic acid was rapid with prostanoid biosynthesis being completed within 4 minutes. The quantitative and qualitative profiles of prostanoids synthesized during four minutes in the NCI-H322 and NCI-H358 cells are summarized below:

CELLS	STIMULUS	$PGF_{2\alpha}$	PGE_2	PGD_2
NCI-H322	5μM A23187	0.29 ± 0.10	3.37 ± 0.58	ND
NCI-H358	5μM A23187	0.29 ± 0.06	2.35 ± 0.07	0.08 ± 0.02
NCI-H322	50μM AA	6.52 ± 1.74	15.70 ± 1.60	1.33 ± 0.32
NCI-H358	50μM AA	6.09 ± 0.54	31.10 ± 2.50	9.44 ± 1.63

These studies clearly indicate that the profiles and quantities of prostanoids synthesized by NCI-H322 and NCI-H358 cells are dependent with respect to the stimulatory agents employed. In addition, we have clearly shown that more than one prostaglandin may be synthesized by these two lung carcinoma cells. The formation of PGE_2, PGD_2 and $PGF_{2\alpha}$ from the common cyclic endoperoxide precursor can occur enzymatically and nonenzymatically. The consistent predominance of PGE_2 formation in arachidonate- and ionophore-stimulated NCI-H322 and NCI-H358 cells strongly suggest that the formation of

PGE_2 is the major enzymatic pathway of metabolism of arachidonic acid in these human lung carcinoma cells. The formation of PGD_2 and $PGF_{2\alpha}$ from PGH_2 may occur at least in part via non-enzymatic degradation as a result of saturation of PGE-isomerase.

HUMAN TUMOR TISSUES

The qualitative and quantitative analysis of prostanoids by high resolution capillary gas chromatography - mass spectrometry described above has also provided the basis for the comparisons of the prostanoid synthesis in tissues from human malignancies and from normal organ tissues in which the tumor occurs. At this time, we have obtained qualitative and quantitative comparisons of prostanoid biosynthesis in twelve human lung carcinomas (including some metastatic tumors) versus normal lung tissue and in three renal cell carcinomas versus adjacent normal renal tissue. In addition to PGE_2, PGD_2 and $PGF_{2\alpha}$, measurable quantities of thromboxane B_2 (TxB_2) and 6-keto-$PGF_{1\alpha}$, have been detected in normal tissue and tumor tissue samples. The number of observations at this time is inadequate for conclusive analysis. Moreover, the number of observations in lung cancer patients is inadequate to obtain a distribution of the different classifications of human lung cancers that is representative of the population of primary lung tumors.

PROPOSED COURSE

The future course of these studies can be subdivided into three major areas. These include the continuation of studies of prostanoid biosynthesis in human tumor isolates in relation to prostaglandin production in normal tissue in which the tumor is resident. These studies will allow us to determine whether direct comparisons of prostanoid profiles (qualitative and quantitative) in normal and tumor tissues will provide us with a direct method for the classification of human tumors in relation to prostaglandin biosynthetic capabilities. The second aspect of future studies is focused upon the qualitative and quantitative profiles of prostanoid production in primary cell cultures derived from human tumors and in established cell lines arising from human malignancies. These studies will allow us to determine whether prostaglandin biosynthesis is a unique characteristic of a group or subclass of human malignancies comprised of certain cell types. The third area will include an evaluation of the in vivo production of prostaglandins in human cancer patients and in laboratory animals bearing tumors resulting from explants of human malignancies (see: Project #Z01 CM 07177-01). The correlation between in vivo prostaglandin production and the classification of human tumors via histopathologic criteria should allow us to determine whether prostaglandin biosynthesis may be elevated in tumors comprised of certain cell types. Our findings may be useful diagnostically and in the classification of human lung carcinomas and other human tumors. These latter studies require the availability of a tetradeuterated analog of the major characteristic urinary matabolite of PGE_2 before such studies can be initiated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07176-01 LETM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Toxicology and Drug Development

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	S. S. Lau	Senior Staff Fellow	LETM, NCI
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	M. R. Boyd	Associate Director	DTP, DCT

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Laboratory of Experimental Therapeutics and Metabolism

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Toxicology and Pharmacology Section

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use stenderd unreduced type. Do not exceed the space provided.)*

The mechanisms by which various chemicals cause nephrotoxicity are poorly understood. It has recently been demonstrated that glutathione (GSH) conjugation with xenobiotics can result in the formation of reactive intermediates and the kidney appears particularly susceptible to the toxic effects of these conjugates. GSH dependent metabolic activation within the kidney probably has greater toxicological significance than that mediated by the cytochrome P-450 dependent mono-oxygenases. This is because of the relatively low activity of renal P-450, the high activity of GSH related enzymes and the rapid turnover of GSH within the kidney. However, little is known of the metabolic and pathological mechanisms by which GSH/cysteine conjugates elicit nephrotoxicity nor of those factors which regulate the generation of potentially reactive thiols from GSH/cysteine conjugates. 2-Bromohydroquinone (2-BHQ) gives rise to a mixture of isomeric mono- and disubstituted GSH conjugates, the latter being a potent nephrotoxin. This is the first example of GSH conjugation to an aromatic substrate leading to toxicity. We have also demonstrated that 2-BHQ can be cooxidized in vitro by prostaglandin synthase to (a) covalently bound material and (b) GSH adduct formation in the presence of GSH. This cooxidation process produced a concentration dependent alteration of prostaglandin synthesis. Furthermore, we have demonstrated that selective transport of 2-BHQ-GSH conjugates into kidney may be mediated by γ-glutamyl transpeptidase (GGT) and that the more extensive renal uptake of the disubstituted GSH conjugate may be partially responsible for its enhanced nephrotoxicity. High levels of GGT are evident in tumors of a variety of tissues, including hepatocellular carcinoma, malignant squamous carcinoma of the skin, squamous cell carcinoma of the buccal pouch epithelium, adenocarcinomas of the lungs and in some mammary tissues. It is therefore conceivable that the studies detailed in this report on the modulation and mechanism(s) of GSH conjugate mediated toxicity might enable a more rational application of such compounds as potential anticancer agents.

Objectives:

1. To delineate the biochemical, pharmacological and toxicological mechanisms of xenobiotic-mediated target organ toxicity.

2. Utilize this knowledge to provide (a) a more rational approach to increase the therapeutic effectiveness of existing conventional anticancer agents while simultaneously reducing their potential for untoward toxic effects and (b) a basis for rational design of target cell-oriented cancer therapy in new drug development.

Current Major Findings:

I. The Effect of Glutathione on Renal Prostaglandin Synthase Mediated Cooxidation of 2-Bromohydroquinone.

Renal necrosis produced by the model toxin, bromobenzene, is mediated in rats by the formation of glutathione (GSH) conjugates of 2-bromohydroquinone (2-BHQ). Neither renal nor hepatic cytochrome P-450 isozymes appear to catalyze the activation of 2-BHQ to a nephrotoxic metabolite since pretreatment with piperonylbutoxide did not protect the animals from 2-BHQ nephrotoxicity. However, we have shown that prostaglandin endoperoxide synthase (PES) prepared from ram seminal vesicles or rat renal papillary homogenates is capable of metabolizing 2-BHQ to reactive intermediates which covalently bind to tissue macromolecules in the presence of arachidonic acid. The cooxidation of 2-BHQ by renal PES caused a concentration - dependent alteration of 6K-$PGF_{1\alpha}$, TxB_2, $PGF_{2\alpha}$, PGE_2, and PGD_2 formation. Concentrations of 2-BHQ as low as 10 μM stimulated prostaglandin formation (2 fold); optimum stimulation occurred at 0.1mM 2-BHQ (3 fold); whereas 1mM 2-BHQ decreased the degree of stimulation. Moreover, 1mM GSH caused a decrease in prostaglandin formation under both control conditions and conditions where prostaglandin synthesis was stimulated by 0.1mM 2-BHQ. GSH also inhibited the covalent binding of 2-BHQ by 90% with a concomitant formation of oxidized GSH (GSSG), an increase in the recovery of 2-BHQ and the formation of 2-BHQ-GSH conjugates. The results suggest that GSH inhibits the PES mediated covalent binding of 2-BHQ by acting both as a one electron reductant in which the reactive intermediate(s) is reduced back to 2-BHQ and by nucleophilic addition to give rise to 2-BHQ-GSH adducts. Moreover, the cooxidation of 2-BHQ by renal PES causes an alteration in arachidonic acid metabolism. The consequences of altered prostaglandin synthesis as a result of xenobiotic cooxidation remain to be established.

II. The Mechanism of 2- Bromohydroquinone Glutathione Conjugate Mediated Nephrotoxicity.

Administration of mono- and di- substituted GSH conjugates as well as the cysteine (CYS) conjugates of 2-BHQ to rats causes elevations in blood urea nitrogen (BUN) and extensive renal necrosis localized in the S_3 segments of the tubules. Whereas a diglutathionyl conjugate caused substantial elevations in BUN, the isomeric monoglutathionyl conjugates caused differentially less toxicity. However, the mechanism(s) by which these conjugates elicit toxicity is unclear. GSH and CYS conjugation has mediates the nephrotoxicity caused by several haloalkanes. The first step in the conversion of GSH conjugates to their corresponding cysteine conjugates is catalyzed by γ-glutamyl transpep-

tidase (GGT). Reactive thiols of the corresponding cysteine conjugates may be formed by the action of a second enzyme, β-lyase. Thus studies were carried out to determine the role these enzymes may play in 2-BHQ-GSH and 2-BHQ-CYS mediated nephrotoxicity. AT-125, a GGT inhibitor, at 10 mg/kg irreversibily inhibited rat kidney GGT activity over 90% in 1 hr and 77% at 24 hrs. Moreover, AT-125 inhibited 2-BHQ-GSH mediated elevations in BUN by 80% but not that of 2-BHQ-CYS. In contrast, neither aminooxyacetic acid (AOA), a β-lyase inhibitor, nor probenecid, an organic anion transport inhibitor, inhibited either 2-BHQ-GSH or 2-BHQ-CYS mediated elevation in BUN. To further delineate the mechanism of reactive intermediates formed from the 2-BHQ-GSH conjugates, isomeric ^{35}S-GSH conjugates of 2-BHQ were synthesized, isolated and purified by HPLC. Covalent binding of the ^{35}S-BHQ-GSH conjugates to rat kidney 10,000g homogenates was in the order 2-Br-6(GSyl)HQ > 2-Br-5-(GSyl)HQ > 2-Br-3-(GSyl)HQ > 2-Br-3,5 or 6(diGSyl)HQ. Moreover, AT-125 (0.4 mM), decreased covalent binding by 25%, 17%, 33%, and 28% respectively. AOA (0.1 mM) inhibited covalent binding by 26%, 10%, 17%, and 17% respectively. Interestingly, ascorbic acid (1.0 mM) inhibited covalent binding by 63%, 87%, 62%, and 28% respectively. The data suggested that the covalent binding is mediated preferentially by redox cycling of the quinone moiety although the function of reactive thiols cannot be excluded.

The reason for the differential nephrotoxicity of the isomeric 2-BHQ-GSH conjugates is unclear but could be due to either differences in the conversion to reactive metabolites or in their uptake to proximal tubular cells. A major determinant of a compound's toxicity is the manner in which it gains access to its target. Knowledge of the means of access is important not only in elucidating the mechanism of toxicity but also in development of therapeutic agents to counteract the toxicity. To investigate the role of renal transport processes in 2-BHQ-GSH mediated toxicity rat kidney slices were prepared. The viability and functional integrity of these slices were demonstrated by their ability to accumulate both organic cations (tetraethylammonium TEA) and organic anions (p-aminohippurate, PAH). 2-BHQ-GSH conjugates inhibited both PAH and TEA accumulation in a time and concentration dependent manner. Moreover, the rate of uptake of 2-Br-3,5 or 6-(diGSyl), 2-Br-5-(GSyl)HQ and 2-Br-6-(GSyl) HQ by kidney slices is 11.9, 4.8, 4.0 and 1.6 nmoles/mg protein/60 min. respectively. GGT activity in intact kidney slices accounted for 5-8% of that in subsequently homogenized kidney slices. AT-125 (0.5 mM) inhibited GGT in intact and homogenized kidney slices by 50% and 92% respectively. These results suggest that accessibility of both substrate and inhibitor to GGT in intact kidney slices is less than that in the cell free preparations. Moreover, AT-125 decreased the accumulation of the isomeric [^{35}S]- conjugates by 49%, 25%, 25%, and 29%, respectively. The data suggest that the transport of 2-BHQ-GSH conjugates into isolated kidney slices may be mediated by GGT within the basolateral membrane and that the more extensive renal uptake of the diGSH conjugate may be partially responsible for the enhanced nephrotoxicity. Furthermore, the kidney specific uptake of 2-BHQ-GSH conjugates by renal GGT followed by the redox cycling of the quinone moiety may be the cause of kidney toxicity. Further investigation is warranted.

III. γ-Glutamyl Transpeptidase Activity And The In Vivo Covalent Binding of ^{14}C-2-Bromohydroquinone

GGT is an ubiquitous enzyme. It is membrane bound and its active site is oriented on the outer surface of the cell membrane. GGT activity is usually

localized to one cell type within the organ and one area of the membrane, where it is highly concentrated. To determine whether differences in tissue distribution of GGT may explain the differences in covalent binding and the selective renal 2-BHQ-GSH mediated toxicity, GGT activity in various rat tissues was measured. The highest activity was found in the kidney (947 U/mg) followed by the pancreas (159 U/mg), seminal vesicles (55 U/mg), intestine (31 U/mg) and bone marrow (5.5 U/mg). Liver GGT was negligible (0.07 U/mg). The in vivo covalent binding of ^{14}C-2-BHQ (0.8 nmol/146 μCi/kg,i.p.) to these tissues was 21.8, 1.5, 1.2, 4.4, 1.8, and 2.6 nmoles/mg respectively. Thus, maximum covalent binding and GGT activity occured in kidney. AT-125 inhibited renal GGT by 76% covalent binding by 74% and 2-BHQ mediated elevations in blood urea nitrogen (BUN) by 73%. Furthermore, covalent binding of ^{14}C-2-BHQ to renal tissue correlated with BUN levels. Although AT-125 inhibited extrarenal GGT activity by 34-66%, it had no significant effect on extra-renal covalent binding. The data suggest that the covalent binding to renal tissue is probably mediated by reactive metabolites of 2-BHQ-GSH conjugates whereas that in extra-renal tissue may be due to 2-BHQ.

IV. Cytotoxicity Study in Human Lung Carinoma Cell Lines

The toxicological significance of the bioactiviation of xenobiotics to toxic intermediates is well recognized. These bioactivations have been shown to be critical for the expression of the cytotoxic and/or carcinogenic potential of many compounds. In the past years, we have selected and maintained several homogenous human lung carcinoma cell lines which differ extensively in their morphology. These cell lines were employed as models to explore the ability of the different human lung cancer cells to activate xenobiotics, especially anti-tumor agents. Both NCI-H69 and NCI-H128 are non-small cell carcinoma cell lines, NCI-H322 and NCI-H358 are well differentiated adenocarcinomas, and NCI-H460 is derived from a large cell carcinoma. It was found that the non-small cell lines have significantly higher oxidative enzyme systems (ie. DEN and ipomeanol oxidase as well as prostaglandin synthase) as compared to the small cell lines. When these cell lines were exposed to mitoxantrone, an anticancer agent, in vitro, a time and dose dependent cytotoxicity (as assessed by measuring leakage of the cytosolic enzyme LDH) was evident in the non-small cell lines. Both small-cell lines were resistant to mitoxantrone. Furthermore, differential cytotoxity after 18 hours exposure to mitoxantrone with these cell lines was observed. IC_{50} of 0.1mM, 0.35mM and 0.45mM in NCI-H460, NCI-H358 and NCI-H322 cells respectively were obtained. Furthermore, inhibitors such as aspirin (0.2 mM) indomethacin (0.2 mM), methimazole (0.2 mM), dicumerol (0.04 mM) piperonyl butoxide (0.5mM) and ascorbic acid (0.5mM) failed to protect against mitoxantrone induced cytotoxicity. The data suggest that factors other than activation of mitoxantrone may play a role in the observed differential cytotoxity of this drug.

Publications:

1. Monks, T.J., Lau, S.S., Highet, R.J. and Gillette, J.R.: Glutathione conjugates of 2-bromohydroquinone are nephrotoxic. Drug Metab. Disp., 13: 553-559, 1985

2. Gillette, J.R., Lau, S.S., Monks, T.J., Satoh, H. and Pohl, L.R.: Chemically reactive metabolites: In Boobis, A.R., Caldwell, J., DeMatteis, F. and Elcombe, C.R. (Eds) : Microsomes and Drug Oxidations, Taylor and Francis Press, 1985, PP 231-237.

3. Monks, T.J. and Lau, S.S. : Reactive intermediates and their toxicologic implications. In de Bruin, A. Gram, T.E. and King, L.J. (Eds): The Biochemical Toxicology of Environmental Agents, 2nd ed. vol.1. General Aspects of the Biodynamics, Metabolism and Elimination of Toxicants. Elsevier Science Publisher, in press.

4. Lau, S.S., McMahon, J.B., McMenamin, M.G., Schuller,H.M. and Boyd, M.R.: Metabolism of arachidonic acid in human cancer cell lines. Cancer Res, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07177-01 LETM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Lung Cancer Research Project

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	T. C. McLemore	Senior Investigator	LETM, NCI
Other:	W. C. Hubbard	Cancer Expert	LETM, NCI
	M. R. Boyd	Associate Director	DTP, DCT
	C. C. Litterst	Pharmacologist	LETM, NCI

COOPERATING UNITS *(if any)*

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Johns Hopkins University School of Medicine (Drs. Eggleston, Liu)
Program Resources, Inc. (Drs. Alley, Fine)

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

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Pharmacology and Toxicology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The development of therapeutic modalities for the treatment of human lung cancer has, to date, been disappointing. Classification of lung tumors with regard to their metabolic potential is particularly important for developing a systematic approach for the identification of active antilung tumor agents. We have, therefore, initiated this project in order to develop a relevant and feasible plan for classification of human lung tumors, as well as for identification of effective new lung cancer treatment modalities. This project will interface the clinical aspects of human lung cancer with basic science and applied science technologies which are currently available in an effort to provide a comprehensive program for characterization of lung tumors based on their morphological, ultrastructural, biochemical, metabolic, and molecular genetic characteristics as well as establish in vitro and in vivo techniques for investigating potential antitumor drugs against primary human lung tumors. The initial results from our laboratories document the feasibility and relevance of this project by demonstrating: (1) considerable variation in prostanoid as well as 4-ipomeanol metabolism among individual fresh human lung tumor specimens, (2) the ability to establish and propagate primary lung tumor cell cultures which will provide material for in vitro drug screening, establishing long term tumor cell lines, and for comparative biochemical studies, (3) the ability to propagate human lung tumors in the lungs of nude mice by a novel intrabronchial implantation method which will allow for improved in vivo propagation and drug screening studies. An ultimate goal of this project is to provide a paradigm for individualized lung cancer characterization and treatment as an improvement over current approaches.

BRIEF DESCRIPTION OF PROJECT

Primary lung cancer has become the major cause of cancer-related adult deaths in the U.S.. Despite advances in treatment of other human cancers, the 5-year survival for lung cancer has remained unchanged (5% to 8%) over the past 20 years. The inadequacies of current approaches for the diagnosis and treatment of lung cancer have led us to an extensive re-evaluation of this disease entity. Recent evidence from the LETM laboratories would suggest that certain ultrastructural and biochemical features might be employed to characterize individual long term lung tumor cell lines. These preliminary data need further investigation especially in fresh human lung tumor specimens. The current lung cancer research proposal interfaces the clinical aspects of human lung cancer with basic science and applied science technologies in an effort to provide a comprehensive program for characterization of lung tumors based on their morphological, ultrastructural, biochemical, molecular genetic and metabolic capabilities as well as establish in vitro techniques for investigating potential antitumor drugs against primary human lung tumors.

OBJECTIVES

I. The establishment of collaborative arrangements with Johns Hopkins University School of Medicine for the purpose of studying a large population of primary lung cancer patients and obtaining well characterized lung tumor specimens for study.

II. The biochemical, metabolic, and molecular genetic characterization of human lung tumors. These presently include:
   1. Prostanoid biosynthesis and metabolism.
   2. 4-Ipomeanol (IPO) metabolism.
   3. Cytochrome P-450 isozymes: metabolism and gene expression.
   4. Cloning of the cytochrome P-450 genes expressed by the human Clara cell tumor cell line NCI-H322.
   5. Conjugating and detoxifying enzymes; metabolism and gene expression.
   6. Cloning of the conjugating enzyme genes expressed by the human Clara cell tumor cell line NCI-H322.

III. The establishment of a system for characterization of human lung tumors based on comparison of their morphological, ultrastructrual, biochemical, metabolic, and molecular genetic properties.

IV. Antitumor drug studies employing primary lung tumor cultures in collaboration with the DTP Lung Cancer Drug Screening Project. This would include:

   1. The comparison between the responses of fresh primary lung tumor cell lines to standard antitumor agents.
   2. The comparison between the in vitro response of individual primary lung tumor cultures and the clinical response of the same patient's tumor to antitumor agents.
   3. The establishment of long term human lung tumor cell lines for use in the in vitro drug screening project.

V. The development of in vivo models in athymic nude mice and rats for fresh human lung tumor propagation and use in drug screening.

1. Intrabronchial (IB) and intrathoracic (IT) implantation model development.
2. Isolated lung perfusion model development.

MAJOR FINDINGS AND RELEVANCE

I. Biochemical and Metabolic Characterization of Human Lung Tumors and Normal Lung Tissue.

a. Prostanoid biosynthesis and metabolism (see Z01 CM 07162 for details)
b. IPO Metabolsim

These preliminary investigations compare the metabolism of IPO (via quantitation of covalent binding of ^{14}C IPO metabolites to cellular macromolecules) in fresh explants of tumor as well as normal human lung tissue obtained at the time of thoracotomy from individual lung cancer patients (PTS) (N=11). Analysis of IPO binding vs. time demonstrated a linear relationship for both tumor and normal tissue over a 45 minute incubation period. A linear relationship also existed between substrate concentration and IPO macromolecular binding for 0-10 mM IPO concentrations in both tumor and normal lung tissue. Inhibition of covalent binding was uniformly observed for tumor and normal tissue by incubation of the tissue for 10 min. with 1mM piperonyl butoxide, boiling the tissue, or placing the tissue on ice at 4°C. Covalent binding of IPO metabolites in normal as well as tumor tissue were similar for the eleven lung cancer pts. studied, with values of 18.3 ± 13.1 and 15.9 ± 14.1, respectively (x ± S.D. pmoles ^{14}C IPO bound/mg protein/30 min; $p > .30$ nonpaired, 2-tailed student t-test). However, considerable interindividual variation in IPO metabolism was noted with normal lung tissue demonstrating a 25-fold interindividual variation in IPO binding while lung tumor tissue demonstrated a 10-fold variation among the different pts. When IPO metabolism was compared for normal and tumor tissue from individual lung cancer pts., no correlation was observed ($r = 0.357$; $p > 0.28$). These preliminary results suggest dysregulation of the metabolism of IPO in lung tumor cells compared with metabolism of this drug in normal lung tissue from the same individual. However, further studies are required to more clearly delineate the mechanisms related to this phenomenon and to ascertain any possible therapeutic implications.

II. In Vitro Propagation of Fresh Lung Tumors

To date, 17 primary lung tumor cell cultures have been established and all 17 have survived. The oldest cultures have now gone through 5 passages but none have reached or passed the crisis stage. The cell types include all four major lung cancer cell types as well as a metastatic prostate and a bladder transitional cell carcinoma. It is anticipated that over the next several months a portion of these early passage cultures will be established as long term cell lines. These can then be employed in in vitro drug investigations as well as for comparative biochemical, metabolic, and molecular genetic studies.

III. New Approaches for In Vivo Propagation of Human Lung Tumors

Conventional in vivo tumor propagation and drug screening models to date have employed predominantly subcutaneous (SC), subrenal capsule, or more recently intraperitoneal implantation of human lung tumors in nude mice. We are exploring the use of a novel approach for the in vivo propagation of human lung tumors, termed IB implantation. This new model provides for implantation within the right mainstem bronchus of nude mice via tracheostomy and subsequent inoculation of tumor cell suspensions through a specially modified intrabroncial catheter. The procedure requires 3-5 min. for completion and has an overall surgical mortality of approximately 5%. Six established tumor cell lines (representing nonsmall cell lung tumors including adenocarcinoma, adenosquamous carcinoma, large cell carcinoma, squamous cell carcinoma and bronchoalveolar cell carcinoma) were implanted either IB or SC using a $1x10^6$ tumor cell inoculum. When the IB technique was employed, 95 ± 8.7% (x ± S.D.) tumor related mortality was observed in mice for the six lung tumor cell lines (N = 23). In contrast, 3.3 ± 5.2% mortality was observed when a $1x10^6$ tumor cell inoculum was employed via SC implantation within 90 day period (N = 65); $p > 0.001$ non-paired, two tailed student t-test). Even when $1x10^5$ tumor cells were utilized via the IB route, 40 ± 32.4% mortality of nude mice was observed compared with 1.7 ± 4% mortality when the same size inoculum was given via the SC route ($p > 0.05\%$). Lung tumor histology and biochemistry in cell cultures and in fresh tumors produced by IB inoculation for the same tumor cell lines were similar. The majority (90%) of the lung tumors propagated by the IB implantation technique were localized in the right lung fields. This new method for in vivo propagation of human lung tumors in the lungs of nude mice, offers several advantages including: (1) lung tumor cells are implanted in the desired organ environment (i.e. the lung), (2) the procedure employs a much smaller tumor inoculum than that required by other models, (3) it provides a potential model for propagation of a high percentage fresh human lung tumor specimens, (4) it offers an attractive and relevant model for in vivo drug testing of potential antilung tumor agents.

FUTURE DIRECTIONS

The studies performed to date indicate that the original objectives proposed for this project are both feasible and relevant and we will therefore continue the project as outlined. In addition, a small group of other relevant human tumors (such as renal cell carcinomas) will be investigated by our current protocols for comparative evaluations. By exploring the relationships among the morphological, ultrastructrual, biochemical, metabolic, and molecular genetic characteristics of individual lung tumors with the in vitro and in vivo response of these tumors to specific antitumor agents, a paradigm for individualized antitumor chemotherapy might be established. This might eventually provide an approach to lung cancer characterization and treatment that is an improvement over existing alternative approaches.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07178-01 LETM

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Carcinogenesis and Developmental Therapeutics

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboretory, and institute affiliation)*

PI:	J. B. McMahon	Cancer Expert	LETM, NCI
Others:	S. S. Lau	Senior Staff Fellow	LETM, NCI
	M. R. Boyd	Associate Director	DTP, DCT

COOPERATING UNITS *(if any)*

LEC, NCI (Dr. Thorgeirsson)
LPET, NCI (Dr. Cooney)

LAB/BRANCH

Laboratory of Experimental Therapeutics and Metabolism

SECTION

Pathology and Ultrastructural Oncology Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreducad type. Do not exceed the space provided.)*

Cell type specificity is a property often shared by agents known to both cause cancer and to cure cancer. Understanding the biology of this target cell specificity may allow us to unravel the carcinogenic process to a point where we can rationally and effectively intervene. This may also facilitate the rational design and development of novel cancer chemotherapeutic agents. We have undertaken a multidirectional approach to carcinogenesis and chemotherapy which involves studies on the cell biology of normal and malignant tissues from both humans and rodents. We have demonstrated that certain human lung cancer cell lines, both of the small cell lung cancer (SCLC) and the non-small cell lung cancer (NSCLC) types, retain the ultrastructural and biochemical characteristics of specific cell types present in the lung in vivo. These cell lines have been characterized extensively and have served as model systems to study many diverse but clinically relevant aspects of human lung cancer cell biology. These cell lines have been utilized in collaborative studies on the mechanisms of actions of several chemotherapeutic agents including arabinosyl-5-azacytosine, merbarone, 2',3'-dideoxycytidine, and BCNU. A well characterized rat alveolar type II cell system has also been developed to study the effects of toxins, carcinogens and anticancer drugs on normal lung cells. Liver cancer cell biology studies which involved chemical transformation of normal rat liver epithelial cells have demonstrated for the first time that there exists a clonal relationship between the two most prominent types of liver cancer, namely hepatocellular carcinomas and cholangiocarcinomas. These studies may lead to the elucidation of the cell types responsible for liver cancer and to more effective therapy. Moreover, the in vitro hepatocarcinogenesis studies have revealed that malignant transformation of rat liver epithelial cells results in the loss of proliferative control exerted by two endogenous polypeptide growth factors, hepatic proliferation inhibitor and transforming growth factor Beta.

OBJECTIVES

Carcinogenesis and cancer chemotherapy are closely related from a cellular standpoint. Both of these complex processes are cell type specific and often represent rare cellular events. A thorough understanding of the biology of normal cell types and their alterations during cellular transformation may therefore allow for rational approaches to both cancer prevention as well as effective cancer chemotherapy. Lung cancer, which is one of the most common diseases, is an excellent candidate for studies involving experimental carcinogenesis and chemotherapy. Extensive studies in rodents have shown that the histogenesis of lung cancer is a sequential multistep process which results in lung tumors comprised of a mixture of functionally and morphologically different cell types. The same tumor heterogenety is often seen in human lung cancer and may partically explain why this disease is one of the least treatable. Pluripotential differentiation of one cell type into another is another property that is common to both the carcinogenesis process and to cells responding to chemotherapy. In order to effectively design therapy for these heterogeneous tumors, the cell biology of the individual cell types and their genotypic and phenotypic interrelationships must be studied in detail. The availability and characterization of defined cell populations isolated from rodents and a large number of human lung cancer cell lines derived from tumors of specific cell types may provide a means to this end.

Liver cancer, at least superficially, appears to be a less complex disease than lung cancer in terms of cellular complexity. However, like lung cancer, liver cancer is one of the most common, most lethal, and least treatable disease. Despite nearly three decades of research little is known about the cell biology of liver cancer with respect to how the proliferation of liver cells is controlled and which cell population is at risk for malignancy. We have attempted to address both these aspects through the use of a well defined rat liver epithelial cell culture system. Studies were initiated to define the factors that are responsible for the control of proliferation of normal liver cells and investigate how these controlling mechanisms are altered during chemical transformation.

METHODS EMPLOYED

Small cell lung cancer (SCLC) cell lines and carcinoids were grown as suspension cultures while nonsmall cell lung cancer (NSCLC) cell lines were maintained as monolayer cultures. All cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum. SCLC cell lines and carcinoids were analyzed for amine precursor uptake and decarboxylation (APUD) features. These cell lines were also analyzed for the modulation of these APUD cell features by biogenic amines. Studies on prostaglandin synthesis and its modulations utilized published RIA techniques modified for tissue culture. Xenobiotic metabolism of the human lung cancer cell lines was monitored by a variety of assays. 4-ipomeanol, a candidate anticancer drug with known target cell specificity toward normal rodent Clara cells, was used as a model substrate for cytochrome P-450 activity. Metabolism of diethylnitrosamine (DEN) was monitored by $^{14}CO_2$ production and covalent binding of radiolabel from ^{14}C-DEN to cell proteins and DNA. Known metabolic inhibitors and inducers were used along with these assays. Assays for specific enzymes involved in xenobiotic metabolism were carried out using standard techniques modified for cultured cells. The cytotoxicity of 4-ipomeanol as well as other candidate

anticancer drugs was monitored by colony forming and soft agar assays systems. Analysis of DNA content, cell size and specific flourescence was done by flow cytometry in collaboration with Dr. Cunningham, Bureau of Biologic, FDA. Ultrastructural analysis under various experimental conditions was done with an analytical electron microscope using the transmission mode at 80 KV. Rat alveolar type II cells were isolated and immunologically identified by methods that we have previously described. Normal rat liver epithelial cells (NRLM) were established from neonatal rat livers by the methods of Herring et al. (In Vitro 19, 576, 1983). These cells were transformed by continuous exposure to a non-cytoxic concentration of aflatoxin B_1 for 10 weeks. They were designated AFB cells when the cell line became tumorigenic in syngeneic rats. This cell line was cloned by limited dilution and 7 cell strains that were produced were injected into syngeneic rats. The morphology of the resultant tumors were analyzed by light and electron microscopy. Frozen sections were stained histo-chemically for gamma glutamyl transpeptidase. Cellular proliferation was assayed by several methods including DNA synthesis, cell number and clony formation. Hepatic proliferation inhibitor (HPI) was isolated by a method previously described.

MAJOR FINDINGS

- The metabolism-dependent covalent binding and cytoxicity of 4-ipomeanol seen in the two human NSCLC cell lines diagnosed as bronchiolo-alveolar carcinomas was demonstrated and was reconfirmed as well using different assay conditions in another NCI Laboratory (FCRF). The metabolism of 4-ipomeanol and diethylnitrosamine by these well differentiated cell lines was oxygen dependent, inducible, substrate concentration and time-dependent.
- The NSCLC cell lines NCI-H322 and NCI-H358 are exceptional in that they possess very little glucuronosyltransferase activity but exhibit appreciable monooxygenase activity.
- Production of PGE_2 in both early and later passages of NSCLC cell line NCI-H460 was linear up to 10 min. Furthermore, maximum production occurred at a substrate concentration of 40μM.
- In a panel of NSCLC cell lines 2'3'-dideoxyycytidine, an inhibitor of HTLV-III infectivity, was shown to be relatively non-toxic and to require metabolic activation.
- BCNU added to isolated type II cells in culture caused marked inhibition of GSSG reductase and a decrease in total cellular glutathione.
- The inhibition of GSSG reductase and depletion of total cellular glutathione was accompanied by an increase in cellular LDH leakage and a decrease in type II cell fatty acid synthesis rates, both markers for cellular toxicity. These data demonstrate that BCNU is toxic to isolated pulmonary type II cells. The biochemical changes caused by BCNU in type II cells mirrored the in vivo effect of BCNU in the lungs of F344 rats.
- TGF β at 20pg/ml caused an 83% inhibition of colony formation of NRLM cells whereas the growth of AFB cells was unaffected by TGFβ at concentrations as high as 10ng/ml. A parallel dose-dependent inhibition of DNA synthesis by TGF β was observed in both primary hepatocytes and NRLM cells at concentrations between 10pg and 10 μg/ml.
- TGF β induced inhibition of the normal NRLM cells was irreversible in nature since treated cells were unable to proliferate upon removal of TGFβ. Furthermore, TGF β induced pronounced morphological changes including cytoplasmic hypertrophy and binucleation.

- TGF β and HPI were both selectively inhibitory towards normal liver epithelial cells and without an effect on chemically transformed liver cells. However, these two 26k dalton polypeptides were found to be chemically, immunologically, and functionally different.
- Chemical transformation of the NRLM cells resulted in a tumorigenic cell population with marked phenotypic and genotypic heterogeneity.
- Single cell cloning of this heterogenous liver cell population followed by transplantation into syngereic animals resulted in the formation of tumors with a wide range of morphological and histochemical characteristics.
- These results suggest that the diploid rat liver epithelial cell culture contained pluripotential "stem" cells which upon transformation could give rise to various tumor cell types.
- Cultures derived from a single hepatic cell can give rise to tumors that are characterized by both hepatocyte and bile duct epithelial cell morphology. Therefore there exists a clonal relationship between hepatocellular carcinoma and cholangiocarcinoma.

SIGNIFICANCE

Lung cancer in man presents vast problems to the scientist both from a clinical standpoint and from basic cell biology. Therapeutic responses are difficult to predict in part because most human lung tumors are heterogeneous and often contain malignant cells of different functional and morphological types. Although recent studies on the various cell types in mammalian lung during normal differentiation and in response to injury have added much to our knowledge, the cellular mechanisms responsible for the generation of heterogeneity and the interconversions of cell types seen in malignancy are poorly understood. Detailed studies on the cell biology of normal and neoplastic lung cells under controlled in vitro conditions may aid in our understanding of these complex processes and help design more appropriate therapies. Moreover, well differentiated cell lines at early passages represent a valuable tool to study cell-type specific biology, biochemistry and pharmacology of identified lung cell types.

Hepatocellular carcinoma (HCC) is probably the most common carcinoma in the world among males. Despite the ease of production of this type of tumor in experimental animals the cellular lineage of HCC and cholangiocarcinoma remains unknown. This is mainly due to the difficulty in identifying the premalignant cells which will ultimately give rise to liver tumors. Experimental results likely to demonstrate direct lineage relationships between liver cell types and cancer may come from studies of well characterized liver cell populations in vitro. Furthermore, such in vitro cell systems may prove to be of value in characterizing the factors that regulate cell proliferation in both normal development and neoplastic disease.

PROPOSED COURSE

Further studies on lung cell biology studies will include the acqusition and characterization of more human cell lines of specific cell types. We plan to use as many early passage cell lines established from human tumors as possible. In this way we can more accurately correlate our observations in vitro with those encountered in vivo. Studies on the chemical and biochemical properties of these cell lines will be expanded both in our laboratory as well as through collaborative projects inside and outside NIH. Similar to what has

been accomplished in the immunological identification of rodent lung cell types through a collaborative effort with Dr. G. Singh at the University of Pittsburg School of Medicine VA Medical Center we are begining a collaborative study with Dr. John Balise of University of South Florida Medical School to use a similar approach to normal and malignant human lung cell types. Studies on liver cell biology will include the further analysis of the cellular lineages of liver cancer using the rat liver epithelial cell system that we have developed and characterized. These studies will include molecular biology studies aimed at determining the critical molecular events in hepatocarcinogenesis and studies on the mechanism of action of the potent hepatic inhibitors HPI and TGF.

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ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Laboratory of Medicinal Chemistry (LMC) was established during FY 1986 to give increased intramural emphasis to (1) the discovery and identification of new anticancer and antiviral drugs from both synthetic and natural sources and (2) the development of analytical methodology appropriate for the quantitation of new drugs in biological fluids and the identification of metabolites. Essentially all projects are collaborative in nature, either among the synthetic and analytical chemists within the Laboratory or between the LMC and the laboratories within the DTP, the NCI or other NIH Institutes.

The discovery by Broder and Mitsuya that 2',3'-dideoxynucleosides (DDO-nucleosides) have anti-HTLV-III activity prompted the LMC to devote a large portion of its FY-86 resources to investigating this area of drug therapy. DDO-cytidine will be the first of these potential anti-AIDS compounds to enter Phase I clinical trial. The LMC analytical chemistry group developed a rapid, simple and sensitive HPLC method for quantitating DDO-C in biological fluids suitable for both pre-clinical and clinical studies. This method has a limit of quantitation of 40 ng/mL. Using this method, the following data were obtained in mice: (1) IV bolus administration of 100 mg/kg showed a terminal phase $t_{1/2}$ of 69 minutes. Urine was the major route of elimination. As predicted from the bolus studies, steady state concentrations of 1 microgram/mL were achieved using continuous infusions. DDO-C was readily absorbed from the gut with oral bioavailabilities of ca 25% observed. DDO-C rapidly penetrated the CNS in monkeys, but only low CSF to plasma ratios (0.03) were achieved. The compound was found to be adequately stable in both human plasma and under the pH conditions encountered in stomach fluids.

A significant amount of synthetic work was carried out in an attempt to exploit the DDO-nucleoside lead to produce even more efficacious analogs. Since DDO-C was the most potent of the DDO-nucleosides discovered by Broder and Mitsuya to be active against the HTLV-III virus, the initial synthetic project undertaken by the LMC was the synthesis of 5-substituted DDO-C analogs. The compounds prepared were chosen to give structure-activity relationship information. DDO-5-azacytidine was anti-HTLV-III active but more toxic than the parent compound. While the 5-methyl and 5-bromo analogs were inactive, 5-fluoro-DDO-C was just as active and potent as DDO-C. This compound is being further evaluated. DDO-5-FU was inactive and toxic.

In an attempt to extend the excellent DNA antiviral properties of the cyclopentenyl (CPE) nucleosides (neplanocin analogs) to AIDS therapy, DDO analogs of neplanocin and CPE-cytidine were prepared and evaluated. These compounds were neither active nor toxic. It is suspected that they are not substrates for deoxycytidine kinase.

Synthetic work in the CPE-nucleoside area was also devoted to non-AIDS related projects. A major objective was realized when the CPE-nucleoside, 3-deazaneplanocin, was synthesized and characterized. Although this compound was

not HTLV-III active, it proved to be a potent inhibitor of at least three RNA viruses by virtue of its inhibition of the enzyme S-adenosylhomocysteine hydrolase. With a 50 picomolar K_i, this compound is 100 times more potent than the best inhibitor of this enzyme previously known. Many improvements in the synthetic chemistry of CPE-nucleosides were discovered. New chemistry was used to synthetize 8-azaneplanocin which had an ID_{50} = 3 micromolar against L1210 cells in vitro.

Earlier LMC synthetic work had produced CPE-cytosine. Continuing evaluation of this compound gave ILS values of 120% against L1210 in vivo, and 290% (50% cures) against ara-C resistant L1210. CPE-C is also a potent inhibitor of CTP synthetase and is active against both DNA (HSV-2, Adenovirus) and RNA (Polio, Dengue) viruses. CPE-uracil is a nontoxic inhibitor of the formation of uridine nucleotides by virtue of its uridine/cytidine kinase inhibition. The antitumor effects of this de novo inhibitor of the pyrimidine pathway in combination with the salvage pathway inhibitor, PALA, is being evaluated by the Laboratory of Biological Chemistry.

Hexamethylene bisacetamide (HMBA) is an agent which causes oncogenic HL-60 cells to differentiate to a nonmalignant phenotype. An HMBA analog synthesis program produced four compounds which were as good or better than the parent compound. However, none were superior enough to be considered as a replacement clinical candidate. Probenecid was determined not to alter the renal excretion of HMBA in rats.

Using new chemistry discovered during the original synthesis of the Phase II drug, dihydro-5-azacytidine, the LMC has prepared a unique intermediate appropriate for constructing oligonucleotides containing 5-azacytosine in defined sequence sites. Model reactions using our new DNA synthesizer indicate that our unusual approach to the incorporation of this hydrolytically unstable base has promise for preparing these potentially specific DNA methyltransferase inhibitors.

Beta-methylene TAD, a stable analog of the active metabolite of tiazofurin, appears to enter cells intact even though it is a dinucleotide. We have recently completed the synthesis of the selenium analog which proved to be twice as potent as the parent compound against L1210 cells in culture.

Tetrahydrouridine (THU), a well-known inhibitor of the enzyme cytidine deaminase, is inactivated by acid and therefore can play no significant role in protecting orally administered ara-C. We have recently synthesized a pyrimidine nucleoside which is acid stable and just as potent as THU as an inhibitor of this enzyme.

Carboacyclic neplanocin analogs containing adenine and guanine were synthesized as acyclovir-related antiviral agents. Carboacyclic G was superior to ara-A but inferior to acyclovir as an inhibitor of HSV-2.

Spiromustine is an LMC drug currently undergoing multi-institutional Phase I/II clinical trials in brain tumor patients. The LMC is cooperating in the Phase I pediatric trial by using its previously developed analytical methodology to study both plasma and CSF pharmacokinetics. The rates of decomposition of a number of new spiromustine analogs in human plasma were also studied. Hydrolytic

stability was found to be influenced by the proximity of the nitrogen mustard group to the hydantoin ring. No analog was discovered which was as active but more stable than the parent drug.

Arabinosyl-5-azacytosine (ara-AC) is an LMC discovered drug which is being developed by the NCI as a clinical candidate. A sensitive and specific HPLC analytical method was developed for this hydrolytically unstable compound in biological fluids which has a 0.4 micromolar limit of quantitation. A bolus dose of 200 mg/kg in mice showed triphasic pharmacokinetics with a terminal $t_{1/2}$ = 52 min. The major hydrolytic metabolite was also determined quantitatively.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 03580-17 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemical Research in the Development of New Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others:	V. E. Marquez	Visiting Scientist	LMC, NCI
	A. Haces	Visiting Scientist	LMC, NCI

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LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of HMBA analogs were synthesized and evaluated as differentiating agents in the in vitro HL-60 cell system. Several compounds were as effective and potent as HMBA but none were clearly superior.

GPO 914-918

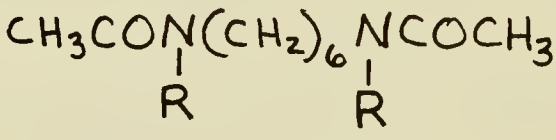
Project Description:

General Objective:

The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DTP screening program, the literature, and biochemical rationale.

Synthesis of Differentiating Agents (Drs. Haces, Breitman, Driscoll). A number of chemicals have been shown to cause certain murine or human tumor cells to differentiate into more mature cells with reduced proliferative properties. The mechanism of action of these materials is unknown. Hexamethylene bisacetamide (HMBA), one of these chemicals, is presently the subject of an NCI-sponsored Phase I clinical trial. It appears that the maximum concentration of HMBA which can be tolerated by humans may be marginal with respect to the concentrations anticipated necessary for differentiation. Therefore, new differentiating agents with higher potencies are required.

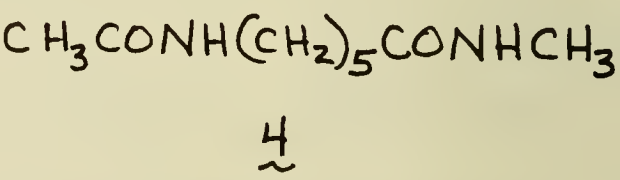
Based on percent differentiation and cell viability data, compounds 2, 3, 4 and 5 are as good or better than HMBA (1) in the HL-60 system in vitro. However, none of these compounds appear to be superior enough to HMBA to propose it as a replacement clinical candidate.



1, R = H (HMBA)

2, R = CH_3

3, R = $COCH_3$



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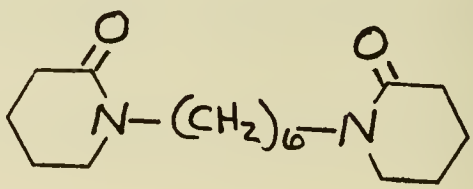


5

PUBLICATIONS:

1. Driscoll, J.S., Johns, D.G. and Plowman, J.: Comparison of the activity of arabinosyl-5-azacytosine, arabinosyl cytosine, and 5-azacytidine against intracerebrally implanted L1210 leukemia. Inv. New Drugs. 3: 331-334, 1985.

2. Haces, A., Driscoll, J.S., Roth, J.S., Heideman, R.L. and Kelley, J.A.: Spiromustine analogs. Relationship between structure, activity and plasma stability. J. Pharm. Sci. 75: 313-316, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06173-01 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Dideoxynucleosides as Potential Anti-AIDS Drugs

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigator.) (Name, title, leboratory, and institute affiliation)*

John S. Driscoll		Chief, LMC	NCI
Others:	V. E. Marquez	Visiting Scientist	LMC, NCI
	C. Tseng	Senior Staff Fellow	LMC, NCI
	J. A. Kelley	Research Chemist	LMC, NCI
	C. H. Kim	Visiting Fellow	LMC, NCI
	A. Goddard	Visiting Fellow	LMC, NCI
	S. Treanor	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Associate Director, Clinical Oncology Program, NCI

LAB/BRANCH

Laboratory Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.4	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this work is the discovery of 2',3'-dideoxynucleosides (DDO) which are superior to known inhibitors of the HTLV-III virus. DDO-5-azacytidine was active but somewhat more toxic than DDO-C. The 5-fluoro analog of DDO-C was just as effective and potent as the parent compound in protecting HTLV-III infected cells. However, the 5-bromo and 5-methyl analogs were ineffective. DDO-cyclopentenyl isosteres (neplanocin analogs) proved to be inactive and non-toxic.

Project Description:

General Objective:

The objective of this project is the discovery of 2',3'-dideoxynucleoside analogs superior to known inhibitors of the HTLV-III virus.

This project includes a significant synthetic effort from project Z01 CM 06174-1 LMC, Cyclopentenyl Nucleoside Isosteres as Potential Antitumor and Antiviral Agents.

Specific Objectives:

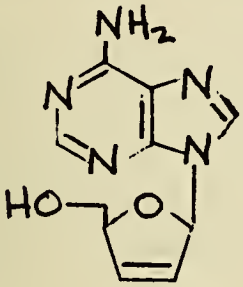
1. Synthesis and evaluation of conventional nucleoside analogs.
2. Synthesis and evaluation of cyclopentenyl nucleoside isosteres (neplanocin analogs).
3. Evaluation of dideoxynucleoside-5'-monophosphates.

Synthesis and Evaluation of Conventional Nucleoside Analogs. The observations of Broder and Mitsuya identifying 2',3'-dideoxy analogs of conventional purine and pyrimidine nucleosides as being active against the HTLV-III virus in vitro provided the lead and rationale for this project. We began by searching the DTP chemistry data base and submitting a number of available compounds for evaluation. Compound 1 was found to have activity at 50 μM concentration (percent protective effect (P): percent cytotoxicity (C) = 57:47) but it was not as effective as dideoxyadenosine (P:C = 100:0).

Because dideoxycytidine (DDO-C) was the most potent, completely protective analog in the DDO-nucleoside series, we have emphasized design and synthetic work in the cytidine series. 2',3'-Dideoxy-5-azacytidine (2) was synthesized with considerable difficulty because of its instability. This compound was active at 10 μM concentration (P:C = 61:17) but it offered no advantage over the parent compound DDO-C. The 5,6-dihydro analog is being prepared. 5-Fluoro-2',3'-dideoxycytidine (3a) was as active as DDO-C at 1 μM concentration, giving essentially complete protection to HTLV-III infected cells. Additional studies at lower concentrations in direct comparison with DDO-C are underway. The 5-methyl (3b) and 5-bromo (3c) analogs of DDO-C were synthesized and were neither active nor cytotoxic. The results from compounds 3a-3c indicate that 5-substituent size, rather than the electronic effect, plays a dominant role in compound activity. The corresponding DDO-5-FU analog (4) was inactive and partially cytotoxic at 30 μM. Studies in the DDO-C series continue.

Synthesis and Evaluation of Cyclopentenyl Nucleoside Isosteres (Neplanocin Analogs). The nucleoside series in which the sugar oxygen atom is replaced by an unsaturated carbon atom is exemplified by the natural product neplanocin (5). The 2',3'dideoxy analog (6a) of 5 was synthesized and neither it nor its 6-chloro analog (6b) was active against the HTLV-III virus. A very difficult synthesis produced the cyclopentenyl analog (7) of DDO-C which was neither active nor toxic. Based on these results, we suspect that these fraudulent nucleosides are not substrates for the respective deoxykinases needed to activate them.

Evaluation of Dideoxynucleoside-5'-Monophosphates. A literature report that 2',3'-dideoxyadenosine-5'-phosphate (8) was capable of entering cells in vitro prompted a study of the effect of 8 on the HTLV-III virus. Initial results indicated that 8 was just as active and potent as DDO-A. When studied at lower concentrations, however, this was not the case and we suspect that phosphatase action produced DDO-A which entered the cell and was active. DDO-C monophosphate was active but not as potent as DDO-C. DDO-T had only moderate activity.



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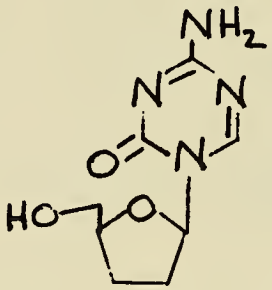

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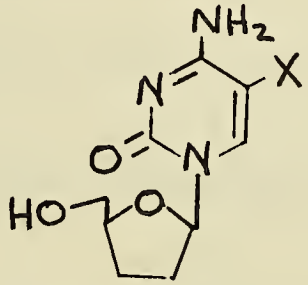

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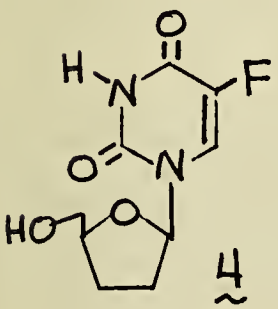
b, X = CH_3
c, X = Br



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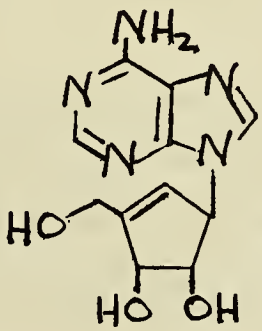

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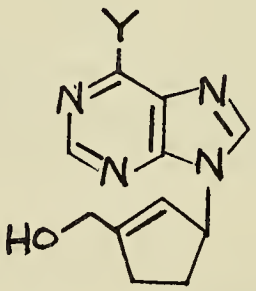
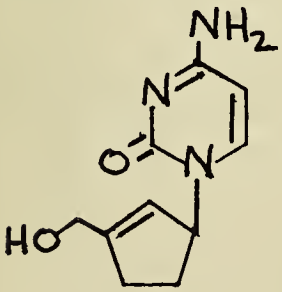


6a, Y = NH_2
b, Y = Cl



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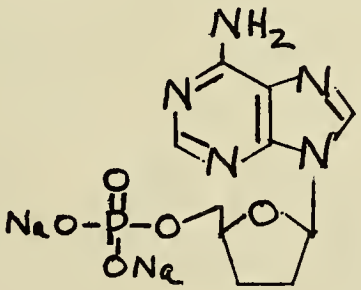

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06174-01 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 chererters or less. Title must fit on one line between the borders.)*

Cyclopentenyl Nucleoside Isosteres as Potential Antitumor and Antiviral Agents.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboretory, end institute affilietion)*

Victor E. Marquez		Visiting Scientist	LMC, NCI
Others:	John S. Driscoll	Chief, LMC	LMC, NCI
	Christopher K.H. Tseng	Staff Fellow	LMC, NCI
	David R. Haines	I.P.A.	LMC, NCI
	Susan P. Treanor	Chemist	LMC, NCI
	Richard W. Fuller	Chemist	LMC, NCI
	Alberto Haces	Visiting Fellow	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pharmacology and Experimental Therapeutics
Laboratory of Biological Chemistry

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

This work has generated a series of novel and known cyclopentenyl (CPE) nucleoside isosteres. The target compounds synthesized comprise: CPE-adenosine (neplanocin A, 4), CPE-3-deazaadenine (3-deazaneplanocin A, 5), CPE-cytosine (11), CPE-uracil (12), CPE-8-azaadenine (8-azaneplanocin A, 6), 2',3'-dideoxy-CPE-cytosine (14), and 2',3'-dideoxy-CPE-adenine (10a). Most of these compounds are endowed with unique antitumor or antiviral activity that appears to be related to the unsaturation present in the carbocycle portion of the molecule. Efforts will continue to exploit this area to its fullest extent using natural and altered aglycone bases. Several new methods of preparation for these compounds have been developed and other alternative syntheses are being explored.

Objectives:

The objective of this work is to exploit the uniqueness of the cyclopentenyl moiety (CPE) as a constituent of nucleoside analogues. A few members of this family of compounds have, so far, produced very powerful antimetabolites in the antitumor and antiviral area, that suggests that the unsaturation present in these carbocyclic nucleosides deserves to be extensively studied. This study is expected to generate a series of potent enzyme inhibitors of key enzymatic reactions that are essential for the progression of neoplasia or viral infectivity.

Major Findings:

CPE-purine analogues (Drs. Tseng, Haines, Marquez, and Mr. Fuller). One of the major goals achieved in this area has been the synthesis of 3-deazaneplanocin A (CPE-3-deazaadenine, 5) which has demonstrated to be the most potent inhibitor of S-adenosylhomocysteine hydrolase when measured against the hamster liver enzyme (K_i = 0.05 nM). In addition, 3-deazaneplanocin A appears to be free of the cytotoxic effects that are characteristic of neplanocin A (Glazer et al., Biochem. Biophys. Res. Commun., 135:688-694, 1986). These two properties are ideal for an antiviral drug and, as expected, 3-deazaneplanocin A at non-cytotoxic concentrations of 50, 25 and 5 μg/ml, displayed potent antiviral activity against dengue II, polio III, and coxsackie A9 viruses. Another purine analogue, 8-azaneplanocin A (6), has been recently synthesized and preliminary results showed good in vitro activity against L1210 cells in culture with an ID_{50} of 3 μM.

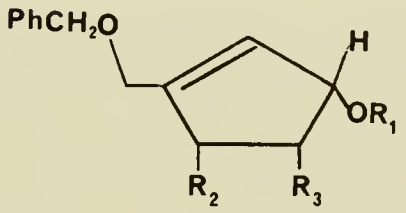
These two carbocyclic purine nucleosides were prepared by a direct displacement approach from the cyclopentenyl α-tosylate 1 and the alkali metal salt of the corresponding purine (Tseng and Marquez, Tetrahedron Lett., 26:3669-3672, 1985).

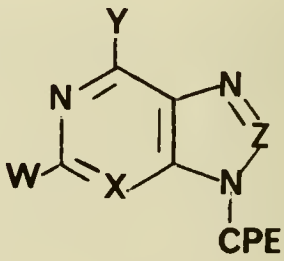
CPE-pyrimidine analogues (Mrs. Treanor and Dr. Marquez). Cyclopentenyl cytosine (CPE-C, 11) was synthesized in a larger scale by our published procedure (Lim et al., J. Med. Chem. 28:1536-1538, 1984) and subjected to a thorough evaluation as an antitumor agent yielding good results in in vivo studies against ip L1210 in mice (1 mg/kg, ip qd 1-9, ILS = 111-122%) and in the resistant variant L1210/araC cell line (1 mg/kg, ip qd 1-9, ILS = 290%, 50% longterm survivors; Moyer et al., Cancer Res., in press). It was also active against P388 leukemia and B16 melanoma in mice but the ILS values were lower. The biochemical effects resulting from treatment with CPE-C suggest potent inhibition of CTP synthetase as the mechanism of action. Direct kinetic experiments with this enzyme await the synthesis of CPE-C triphosphate. CPE-C also showed potent antiviral activity against DNA (herpes simplex II and adenovirus 7) as well as RNA (polio type III and dengue type II) viruses. Against herpes simplex II, it had a viral rating (VR) of 2.3 and a MIC_{50} = 2.7 μg/ml which is between that of araA and acyclovir (positive controls). Another pyrimidine analogue with promising biological activity is cyclopentenyl uridine (CPE-U, 12) which at a noncytotoxic dose of 1000 mg/kg was capable of inhibiting the formation of Z01 CM 06174-1 LMC uridine nucleotides (> 90% in mouse liver) as a consequence of its inhibitory action against uridine-cytidine kinase. This compound will serve to test the usefulness of inhibiting the salvage pathway when used concomitantly with de novo inhibitors of pyrimidine biosynthesis (e.g. PALA).

General Chemistry (Drs. Tseng, Haces, Haines, Marquez and Mrs. Treanor). The highlighted biological results in this area have prompted us to renew our efforts in the synthesis of other CPE analogues of important nucleoside metabolites and antimetabolites. Syntheses of the following compounds have been achieved: ara-CPE-C (17), CPE-T (15), 3'-dCPE-T (16). The use of the direct displacement approach discovered and reported last year has proven to be very effective particularly with purines. The following compounds were prepared by this procedure: CPE-6-chloro-2-aminopurine (8), CPE-guanine (7), and CPE-3-aminotriazolotriazole (9).

New methods of coupling using the Mitsunobu reaction allowed use of the alcohol 2 directly without the need of activation via the tosylate. Such an approach led to the synthesis of 13. In addition, different conditions applied to the condensed products (protected CPE-nucleosides) permitted the selective removal of blocking groups (benzyl and isopropylidene).

Finally, the discovery of the activity of dideoxynucleosides against AIDS (see project Z01 CM 06173-1 LMC) prompted the synthesis of 14 and 10a. These two compounds were prepared from 11 and 4 (neplanocin A) following a two-step Barton reduction that employed the thiocarbonylimidazole/tri-n-butyltin hydride sequence. The procedure worked well for CPE-C but the yields were low when neplanocin A was employed. An alternative approach to 10a is being attempted through the use of the Mitsunobu reaction starting with the recently prepared alcohol 3. Conversion of 10b to the final target 10a is in progress.





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2, R_1 = H; R_2,R_3 = 

3, R_1 = H; R_2 = R_3 = H

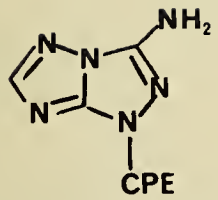
4, W = H; X = N; Y = NH_2; Z = CH

5, W = H; X = CH; Y = NH_2; Z = CH

6, W = H; X = N; Y = NH_2; Z = N

7, W = NH_2; X = N; Y = OH; Z = CH

8, W = NH_2; X = N; Y = Cl; Z = CH



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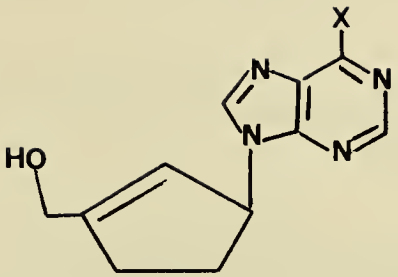
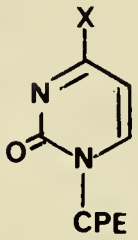


10a, X = NH_2

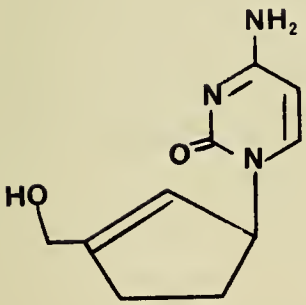
b, X = Cl



11, X = NH_2

12, X = OH

13, X = H



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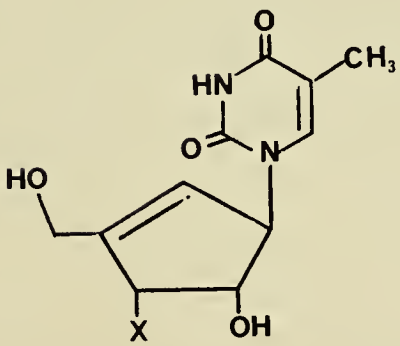
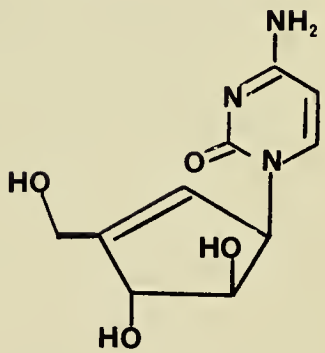


15, X = OH

16, X = H



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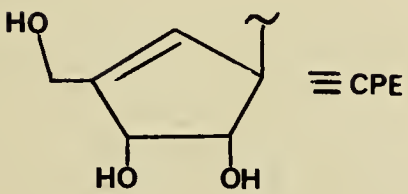


≡ CPE

PUBLICATIONS

1. Glazer, R.I., Knode, M.C., Lim, M-I. and Marquez, V.E.: Cyclopentenyl cytidine analogue: an inhibitor of cytidine triphosphate synthesis in human colon carcinoma cells. Biochem. Pharmacol., 34: 2535-2539, 1985.

2. Moyer, J.D., Karle, J.M. Malinowski, N., Marquez, V.E., Salam, M.A., Malspeis, L., Cysyk, R.S.: Inhibition of uridine kinase and the salvage of uridine by modified pyrimidine nucleosides. Mol. Pharmacol., 28: 454-460, 1985.

3. Tseng, C.K.H. and Marquez, V.E.: An improved method of synthesis of neplanocin and related cyclopentenyl-containing nucleosides. Tetrahedron Lett., 26: 3669-3672, 1985.

4. Glazer, R.I., Hartman, K.D., Knode, M.C., Richard, M.M., Chiang, P.K., Tseng, C.K.H., Marquez, V.E.: 3-Deazaneplanocin A: A new and potent inhibitor of S-adenosylhomocysteine hydrolase and its effects on human promyelocytic leukemia cell line HL-60. Biochem. Biophys. Res. Comm., 135: 688-694, 1986.

5. Marquez, V.E. and Lim, M-I.: Carbocyclic Nucleosides. Medicinal Research Reviews. 6: 1-40, 1986.

6. Glazer, R.I., Cohen, M.B., Hartman, K.D., Knode, M.C., Lim, M-I., Marquez, V.E.: Induction of differentiation in the human promyelocytic leukemia cell line HL-60 by the cyclopentenyl analogue of cytidine. Biochem. Pharmacol. (in press).

7. Moyer, J.D., Malinowski, Treanor, S.P., Marquez, V.E.: Antitumor and Biochemical Effects of Cyclopentenyl Cytosine. Cancer Res. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06175-01 LMC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*
Synthesis and Properties of Oligonucleotides Containing 5-azacytosine Residues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Victor E. Marquez	Visiting Scientist	LMC, NCI
Others: Amanda Goddard	Visiting Fellow	LMC, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the spece provided.)*

This work intends to achieve the synthesis of 5-azacytosine-containing DNA fragments which are designed as specific inhibitors of DNA methyl transferase. After genome insertion, the relationship between the location of the 5-azacytosine residue and the base sequence of the oligomer is expected to confer the resulting molecule sufficient selectivity to allow activation of those specific genes for which DNA methylation is inhibited.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

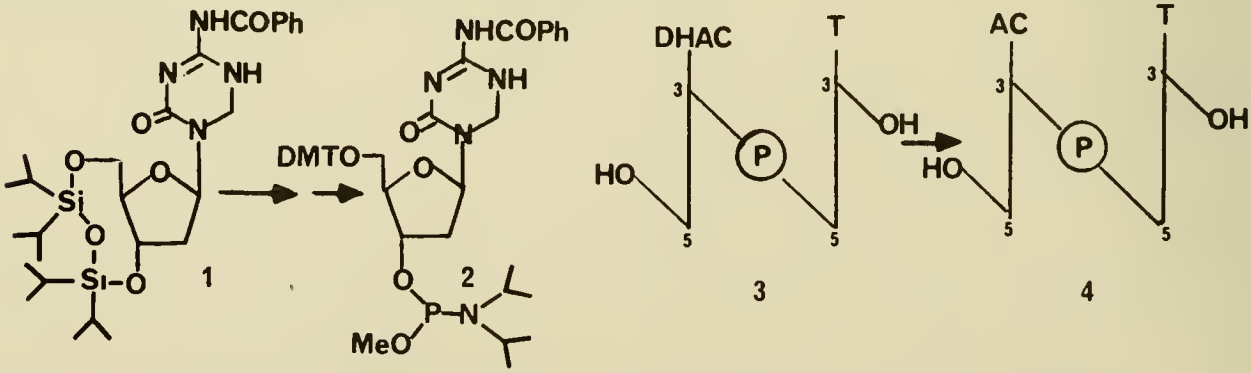
5-Azacytidine (5-azaCR) and 5-aza-2'-deoxycytidine (5-azaCdR) are capable of activating specific genes through the inactivation of DNA methyltransferase activity. Demonstrable hypomethylation of CpG sites in the activated genes results when a minor fraction of cytosine residues are replaced by 5-azaC. Since cellular incorporation of 5-azaCdR appears to be a random process, we became interested in building DNA segments (oligonucleotides up to 40 units long) where the site of cytosine replacement is known. The long term goal of this project is to eventually activate certain genes through the specific incorporation of 5-azaC residues in their promoter regions. These synthetic oligonucleotides would have to be incorporated in the cell's genome through molecular engineering techniques in order to exert their biological effects.

Major Findings.

This project began recently and a series of basic problems had to be addressed first: a) selection of the oligonucleotide sequence; b) selection of the cytosine residues to be replaced by 5-azaC; c) synthesis of the 5-azaCdR monomer suitably protected and compatible with the chemistry of automatic DNA synthesizers and d) synthetic strategies to overcome the known instability of the 5-azaC residue. A sequence known for its substrate properties for DNA methylase was chosen: 5'TCGACCCCCCCCCCCCCCCGGGTCTAG-3'. According to Weissbach et al. (Nucleic Acid Res., 13:3479-3494, 1985) this molecule is an excellent substrate for DNA methylase which incorporates a methyl group specifically at C-19. We postulate that substitution of 5-azaC for cytosine at position 19 should produce a potent inhibitor of the methylase reaction. Other oligonucleotide sequences typical of promoter regions will be later selected for synthesis.

Being aware of the extreme instability of the 5-azaC residue, particularly to base, the dihydro-5-azaC (DHAC) precursor phosphoramidite 2 was sought as a target. After completion of the DNA synthesis (the final step requiring basic conditions) the stable dihydro-5-azaC moiety will be converted to 5-azaC through the use of a mild oxidant (Kelley et al., Anal. Biochem., 103:203-213, 1980).

This project has progressed up to the stage of the four-step synthesis of 1 which is a direct precursor of 2 (three more steps required). Compound 2 will be used to prepare dimer 3 by conventional methods and will serve as a model to test the use of the silylation-mediated oxidation to 4.



DMT = dimethoxytrityl, DHAC = dihydro-5-azaC, AC = 5-azaC, T = thymine

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06176-01 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Enzyme Inhibitors as Potential Anticancer and Antiviral Drugs.

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, end institute affiliation)*

Victor E. Marquez		Visiting Scientist	LMC, NCI
Others:	John S. Driscoll	Chief, LMC	LMC, NCI
	David R. Haines	I.P.A. Visiting Professor	LMC, NCI
	Christopher K.H. Tseng	Visiting Fellow	LMC, NCI
	Chong-Ho Kim	Visiting Fellow	LMC, NCI
	Richard W. Fuller	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pharmacology and Experimental Therapeutics
Laboratory of Biological Chemistry

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	1.9	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Usa standard unreduced type. Do not exceed the space provided.)*

The scope of this project is to design and synthesize mechanism-based inhibitors of enzymatic reactions that are critical for the growth of neoplastic cells or viral replication. The resulting compounds are intended to be used as therapeutic agents and research probes. The following topics are of current interest: 1) Dinucleotide analogues of NAD as inhibitors of IMP dehydrogenase, 2) Acid-stable inhibitors of cytidine deaminase intended for oral use, 3) Synthesis of diazepinone nucleosides as dCMP deaminase inhibitors, 4) Synthesis of phosphonate analogues of the 2',5'-oligoadenylate trimer as stable inducers of interferon production, 5) Synthesis of purine phosphonates as inhibitors of purine nucleoside phosphorylase (PNP), 6) Synthesis of carbocyclic nucleosides as potential antiviral agents.

PHS 6040 (Rev. 1/84) GPO 914-918

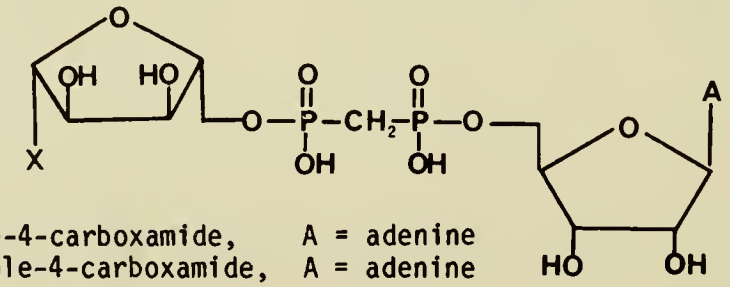
Objectives:

The objective of this project is the exploitation of recent biochemical findings concerning enzymatic mechanisms that are enhanced in rapidly proliferating or in viral infected cells, in order to design appropriate inhibitors for key enzymes. A suitably altered substrate (mechanism-based inhibitor) is selected as a candidate target and synthesized. The selected candidates are designed as reversible transition-state inhibitors or irreversible (K_{cat}) suicide inhibitors. A correlation between structure and activity in the series of inhibitors prepared serves to delineate the course of future synthetic work.

Major Findings:

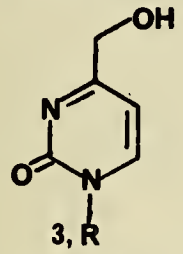
Dinucleotide Analogues of NAD as Inhibitors of IMP Dehydrogenase (Drs. Marquez, Tseng and Mr. Fuller.

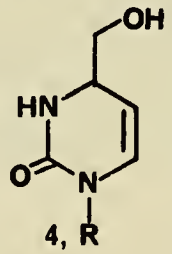
As mentioned in last year's report, the β-methylene TAD analogue 1 was synthesized and demonstrated to be a hydrolytically stable analogue of TAD with equipotent inhibitory activity against IMP dehydrogenase. This year it has been shown that 1 at 50 μM concentration, significantly reduces guanine nucleotide pools in sensitive P388 cells in culture to levels comparable to those achieved with 2.5 μM of tiazofurin. (Marquez, et al., J. Med. Chem (in press). This result suggested that β-methylene TAD was, as expected, entering the cell intact and inhibiting IMP dehydrogenase. TAD, on the contrary, was cleaved under similar conditions by phosphodiesterases to tiazofurin monophosphate and AMP. After further hydrolysis to tiazofurin this compound is intracellularly assembled back to TAD in sensitive cells. A critical test to demonstrate the potential of 1 as a drug, indicated that this compound at 50 μM produced drastic reductions of guanine nucleotides (GMP, 3%; GDP, 24%; GTP, 5% of control values) even in tiazofurin-resistant P388 cells which lack the capacity to form TAD from tiazofurin. This demonstrated that 1 was not only hydrolytically stable but capable of entering the cell. Recently, we have completed the synthesis of the selenium analogue, β-methylene SAD (2) by a similar approach coupling adenosine 5'-(α,β-methylene) diphosphate with the acetonide of selenazofurin in the presence of dicyclohexylcarbodiimide. The rationale behind this synthesis was to exploit the ten-fold difference in potency as IMP dehydrogenase inhibitor that exists between TAD and SAD in favor of the selenium analogue. If such difference was to be maintained for the β-methylene analogues, it was expected that the effective dose (ID_{50}) of β-methylene SAD would be reduced to approximately 5 μM. Preliminary results indicate that the ID_{50} dose is reduced to approximately one half (ID_{50} = 23 μM) indicating perhaps that transport of these charged molecules may be a limiting factor. A series of related structure-activity studies will continue with emphasis on improving transport.

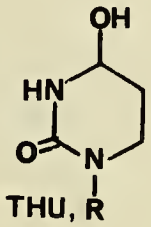


1, X = thiazole-4-carboxamide, A = adenine
2, X = selenazole-4-carboxamide, A = adenine

Acid-Stable Inhibitors of Cytidine Deaminase (CDA) Intended for Oral Use (Drs. Kim and Marquez). The compounds 3 and 4 were synthesized last year as part of an ongoing effort in developing CTP synthetase inhibitors. These compounds, however, proved to be rather inocuous and non-cytotoxic when tested in vitro against L1210 leukemia. Due to the structural similarities that exist between the transition-states of CTP synthetase and cytidine deaminase (CDA), the compounds were tested as CDA inhibitors against the mouse kidney enzyme. Compound 4 proved to be as potent as tetrahydrouridine (THU) in this system with a K_i of 0.4 μM. This compound, however, has the additional advantage that contrary to THU it is highly UV active, which facilitates its detection, and virtually acid stable. THU as we have reported (Kelley et al., J. Med. Chem., submitted) undergoes a very facile and rapid transformation into the inactive ribopyranoside form. On the contrary, compound 2 at pH values that isomerized THU more than 90% to the ribopyranoside form, remained 84% as the intact and active ribofuranoside, as judged from NMR studies. It is believed that the unsaturation present in 4, which is absent in THU, is responsible for its increased acid stability. Compound 2 will be of interest in studies with orally administered araC.

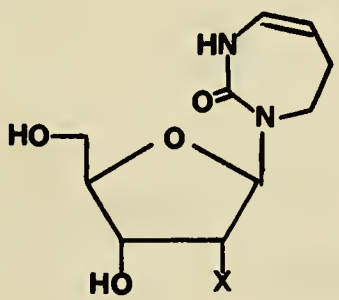






R = β-D-ribofuranosyl

Synthesis of Diazepinone Nucleosides as dCMP Deaminase Inhibitors (Drs. Kim and Marquez). Based on the literature evidence that dTHU (2'-deoxytetrahydrouridine) effectively inhibits dCMP deaminase after its phosphorylation to the monophosphate level (see Boothman et al., Molecular Pharmacol., 27: 584-594, 1985), we selected our most potent diazepinone nucleoside cytidine deaminase (CDA) inhibitor 5 (K_i = 0.08 μM) as a starting point towards the synthesis of the corresponding 2'-deoxyriboside analogue 6. This compound was prepared after the simultaneous protection of the 3' and 5'-hydroxyl groups of 5 with 1,3-dichloro-1,1,3,3-tetra-isopropyldisiloxane, followed by the reaction of the remaining free 2'-hydroxyl group with thiocarbonyl-bis-imidazole and reduction with tri-n-butyltin hydride (Barton reduction). Removal of the protective groups afforded 6 in good yields. This compound showed equivalent inhibition of CDA as the ribofuranoside 5. Studies aimed at determining the utility of 6 as a dCMP deaminase inhibitor are in progress.

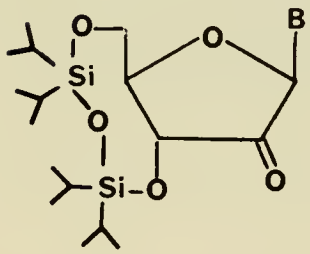


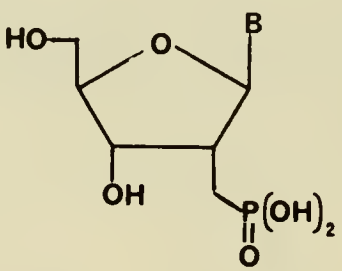
5, X = OH

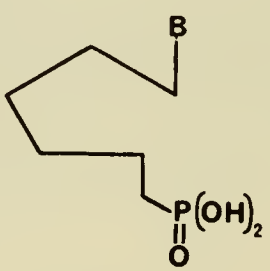
6, X = H

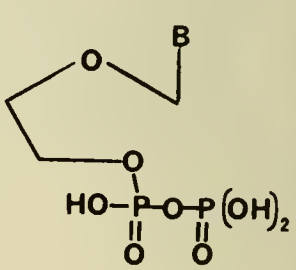
Synthesis of Phosphonate Analogues of the 2',5'-oligoadenylate Trimer as Stable Inducers of Interferon Production (Drs. Tseng and Marquez). This project remained latent for the most part of the year due to our heavy commitment to the cyclopentenyl nucleoside project (Z01 CM 06174-1). Efforts to obtain the monomer 8 from the ketoadenosine precursor 7 are now being explored in order to take advantage of the expected regioselectivity of 7 which allows nucleophilic attack exclusively from the α-side. Synthesis of 8 was accomplished last year, but the stereochemistry at C-1' and C-2' still remain uncertain.

Synthesis of Purine Phosphonates as Inhibitors of Purine Nucleoside Phosphorylase (Drs. Tseng and Marquez). The monomeric phosphonate 8 from the previous work has the potential of being exploited as a transition-state inhibitor of purine nucleoside phosphorylase (PNP). If the base B is changed to hypoxanthine, as in compound 9, the compound will resemble two of the most powerful PNP inhibitors reported to date. Recent studies have shown that acyclovir diphosphate (11, B = guanine) is one of the most potent inhibitors of the PNP (K_i = 0.51 μM) the enzyme from human erythrocytes (Tuttle and Krenitsky, J. Biol. Chem., 259:4065-4069, 1984). Since from transition-state (TS) theory it is expected that the attacking phosphate and the departing base will be in close proximity during the PNP reaction, the molecule of acyclovir diphosphate is probably "folded" and therefore acting as a multisubstrate TS analogue inhibitor. Parks et al. (Biochem. Pharmacol., 35:133-136, 1986) recently utilized this concept to prepare several hypoxanthine phosphonate analogues which were expected to behave as acyclovir diphosphate. One of this analogues, compound 10 (B = hypoxanthine), produced significant inhibition of the PNP reaction with a K_i of 0.9 μM. When our phosphonate 9 is compared with a folded version of 10 and 11, it shows that the phosphonate (or phosphate in acyclovir) and the base are in a similar spatial relationship that is expected from TS theory. The advantage of 8 over its acyclic counterparts is that 8 has fewer degrees of freedom and could interact more readily with the receptor having the correct disposition of functional groups. The inhibitory activity of 9 against PNP will be studied soon. A potent inhibitor of PNP will be of great interest since PNP provides a key step for guanine and hypoxanthine salvage and catabolism.



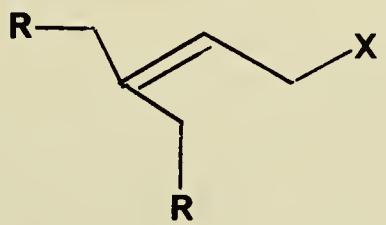






7, B = adenine 8, B = adenine 9, B = hypoxanthine 10, B = hypoxanthine 11, B = guanine

Synthesis of Carboacyclic Nucleosides as Potential Antiviral Agents (Drs. Haines, Tseng, and Marquez). The significant antiviral effect of 3-deazaneplanocin described in project Z01 CM 06174-1, combined with the well known antiviral properties of acyclic nucleosides, prompted us to perform a molecular dissection of cyclopentenyl purines (i.e. neplanocin A and CPE-guanine) where the carbon-2' of the carbocyclic is excised. Through this operation the resulting molecules 13 and 14 become structurally analogous to the well known antiviral agents acyclovir and dihydroxypropoxymethyl guanine (DHPG). The syntheses of these analogues proceeded from 1,3-dibenzyloxyacetone which was used in a Wittig condensation with triethyl phosphonoacetate to give the corresponding α, β-unsaturated ester. Reduction of the ester to the allylic alcohol, followed by conversion of the alcohol to the chloride 12, allowed based catalyzed condensations with different aglycones to generate the adenine and guanine carboacyclic nucleosides 13 and 14 after debenzylation. The synthesis of 13 was direct whereas that of 14 proceeded via the 2-amino-6-chloropurine intermediate. These compounds showed antiviral activity against herpes simplex II. In particular, compound 14, was superior to araA (positive control, VR = 1.2, MIC_{50} = 26.8 μg/ml) with a viral rating (VR) of 1.5 and a MIC_{50} = 65.6 μg/ml. However, acyclovir in this test system proved to be superior to 14 with a VR of 5.1 and MIC_{50} = 4.3 μg/ml. The adenosine analogue was less potent and when tested as a possible inhibitor of S-adenosylhomocysteine hydrolase it showed virtually no activity against this enzyme as opposed to its cyclic analogue neplanocin A.



12, X = Cl, R = OCH_2Ph

13, X = adenine, R = OH

14, X = guanine, R = OH

PUBLICATIONS

1. Monks, A., Marquez, V.E., Mao, D.T., Cysyk, R.K.: Inhibition of nucleoside transport by 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin) and related analogues. Cancer Lett., 28: 1-8, 1985.

2. Marquez, V.E., Tseng, C.K.H., Gebeyehu, G., Cooney, D.A., Ahluwalia, G.S., Kelley, J.A., Dalal, M., Fuller, R.W., Wilson, Y.A., Johns, D.G.: Thiazole-4-carboxamide adenine dinucleotide (TAD). Analogues stable to phosphodiesterase hydrolysis. J. Med. Chem. (in press).

3. Kim, C-H., Marquez, V.E., Mao, D.T., Haines, D.R.: Synthesis of pyrimidin-2-one nucleosides as acid-stable inhibitors of cytidine deaminase. J. Med. Chem. (in press).

4. Ahluwalia, G.S., Cooney, D.A., Marquez, V.E., Jayaram, H.N., Johns, D.G.: Studies on the mechanism of action of Tiazofurin (2-β-D-ribrofuranosyl-thiazole-4-carboxamide) VI. Biochemical and Pharmacological Studies on the Degradation of Thiazole-4-carboxamide Adenine Dinucleotide (TAD). Biochem. Pharmacol. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 03581-17 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Analytical Chemistry of New Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James A. Kelley		Research Chemist	LMC, NCI
Others:	John S. Driscoll	Laboratory Chief	LMC, NCI
	Jeri S. Roth	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Lab. Pharmacol. Exp. Therap., Lab. Exp. Therap. Metab., DTP, DCT; Clin. Pharmacol. Branch, Pediatrics Branch, COP, DCT; Invest. Drug Branch, CTEP, DCT; Surgical Neurology Branch, NINCDS

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.8	0.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the research and development of analytical methods which are used to: (1) establish the structure and purity of new anti-tumor agents and their metabolites, (2) determine physical and chemical properties of new anticancer drugs, (3) quantitate drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmacokinetics, and (4) study reaction mechanisms of potentially useful synthetic transformations. Mass spectrometry, gas chromatography and high-performance liquid chromatography, either alone or in combination, are emphasized techniques. Compounds of current interest are cytidine analogs, cytidine deaminase inhibitors, modified nucleosides, oligonucleotides, nitrogen mustards and differentiating agents.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

General Objectives:

The objective of this project is the research and development of analytical techniques for establishing the structure and purity of new anticancer drug candidates, determining their important physical and chemical properties, elucidating structures of metabolites of new antitumor agents, measuring these drugs and their metabolites in physiological samples and studying reaction mechanisms. Mass spectrometry (MS), gas chromatography (GC), high-performance liquid chromatography (HPLC) and the combination of these techniques are the emphasized methods. Other analytical methods such as NMR, UV and IR spectroscopy are also employed.

Specific Objectives:

1. Analytical methods development and preclinical pharmacology for arabinosyl-5-azacytidine (Ara-AC).

2. Plasma and CNS pharmacokinetics of spiromustine in a pediatric Phase I clinical trial.

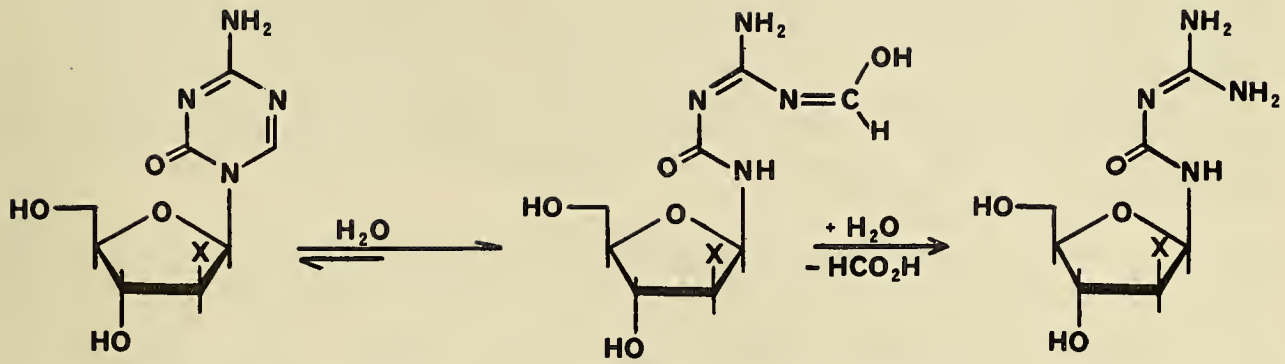
3. Effects of probenecid on hexamethylene bisacetamide plasma elimination.

4. Synthetic and collaborative project support.

Major Findings:

1. Analytical Methods Development and Preclinical Pharmacology for Arabinosyl-5-azacytidine (Ara-AC):

a. Analytical Methods Development (Dr. Kelley, Ms. Roth): Ara-AC (1, NSC 281272) is a new synthetic nucleoside which combines the structural elements of two established antitumor agents, Ara-C (the arabinose sugar) and 5-AC (the triazine base). Chemically Ara-AC behaves like 5-AC, while it most closely resembles AraC in its antitumor and other biological properties. A reliable, sensitive and specific HPLC assay has been developed for the measurement of Ara-AC in biological fluids. This assay utilizes reverse phase HPLC with tandem C_8 and C_{18} cartridge columns and UV detection at 240 nm. 2'-Deoxy-5-azacytidine (2), an analog with similar aqueous stability (Scheme I), is employed as an internal standard. Ultrafiltration and solid phase extraction with a phenyl boronic acid column are used to remove plasma proteins and endogenous ribosides. This assay allows separation and measurement of both Ara-AC and its initial hydrolytic decomposition product 3. For human plasma a limit of quantitation of 0.1 µg/ml (0.4 µM) could be attained when using a 1.0 mL plasma sample; for mouse plasma, the limit of quantitation was 0.25 µg/ml with a 0.5 mL plasma sample.



1, X = OH (ara-AC)
2, X = H (DAC)

3, X = OH (AGU-CHO)
4, X = H

5, X = OH
6, X = H

b. Bolus Dose Kinetics in Mice (Drs. Kelley, Litterst, Ms. Roth): The plasma kinetics of Ara-Ac in male BDF_1 mice was determined after a single i.v. bolus dose of 200 mg/kg. Plasma elimination was triphasic with a total body clearance of 17.1 mL/(min-kg) and a terminal phase half-life of 52 min. The plasma concentrations of the initial hydrolytic product 3 could also be followed for about 3 hr after drug administration. The relative concentration of 3 gradually increased to an apparent equilibrium value of about 0.15 Ara-AC molar equivalents at 1 hr and then parallelled the elimination of the parent compound.

2. Plasma and CNS Pharmacokinetics of Spiromustine in a Pediatric Phase I Clinical Trial (Drs. Kelley, Heideman, Poplack, Ms. Roth): Spiromustine (spirohydantoin mustard, NSC 172112) is a new lipophilic alkylating agent designed for the treatment of central nervous system (CNS) tumors. This compound is currently being evaluated in Phase I and II clinical trials. Among these is a cooperative pediatric Phase I study (NCI, Bethesda; Children's Hospital, Philadelphia; Children's Hospital National Medical Center, Washington, D.C.) in which patients, 4 to 18 years of age, have been administered spiromustine as an i.v. bolus dose on a weekly x 3 schedule every 28 days. Patients with a variety of CNS and non-CNS solid tumors have received 4.5-9.5 mg/m^2 spiromustine per course of treatment. As in the adult Phase I clinical trials, neurotoxicity is significant and has been the major toxicity encountered. Serial plasma samples from 6 i.v. bolus courses of therapy (4 patients) have been analyzed using a previously developed wide-bore fused silica capillary column GC - nitrogen phosphorus detector (NPD) assay, and the pharmacokinetic parameters have been calculated (Table 1). A two-compartment open model where the $t_{1/2}$ for the terminal phase is only 13.9 ± 3.9 min (n = 6) appears to adequately define the plasma elimination

curve and coincides closely with the results of an adult Phase I study. Administration of spiromustine as a short infusion (15-16 min) to two of these same patients at 5.5 and 6.5 mg/m^2, respectively, further validated the above pharmacokinetic model. The average measured plasma concentration (C_p) at steady state showed that the very rapid plasma clearance of this drug had been reliably estimated by the bolus dose kinetics. Spiromustine could also be measured in the cerebrospinal fluid (CSF) of two patients within 10 min after drug administration. However, levels were at the limit of quantitation of the GC-NPD assay and the resulting CSF to plasma ratio of 0.035 - 0.046 was surprisingly low. This may be due in part to extensive plasma protein binding, if the results indicating 91 ± 3% binding of 1 µg/mL spiromustine in human plasma at 3° can be extrapolated to an in vivo system at 37°.

Table 1. Pharmacokinetic Summary of Spiromustine Pediatric Phase I Clinical Trial

Patient	Dose (mg/m^2)	Cycle/Course	Peak[a] C_p (ng/mL)	$t_{1/2}(\alpha)$ (min)	$t_{1/2}(\beta)$ (min)	MRT[b] (min)	Cl_{TB}[c] (mL/min^2)	V_{dss}[d] (L/m^2)
P.C.	5.5	1/1	215	1.5	9.3	5.5	2455	20.9
	5.5	1/2	240	2.1	12.8	5.9	3026	27.9
R.H.	5.5	1/1	546	1.5	10.3	4.4	1666	10.6
	5.5	1/2	887	2.2	18.3	5.2	1103	10.9
S.L.	6.5	1.1	1552	0.7	13.8	3.6	1627	12.5
A.S.	7.5	1.1	552	2.6	18.7	5.5	2408	24.3

[a] measured; time of first blood sample varies
[b] mean residence time
[c] total body clearance
[d] volume of distribution at steady-state

3. Effect of Probenecid on Hexamethylene Bisacetamide Plasma Elimination (Drs. Kelley, Litterst, Chun, Ms. Roth). Probenecid blocks the tubular secretion of drugs that are weak acids. Thus smaller doses of these drugs have to be given when probenecid is co-administered to achieve the same plasma concentration as when they are given by themselves. Since substantial amounts (grams) of hexamethylene bisacetamide (HMBA) must be administered as a continuous i.v. infusion to maintain the required millimolar plasma levels, the potential benefit of probenecid is obvious. Whether probenecid could enhance plasma concentrations of HMBA or alter its renal clearance was investigated in male Sprague-Dawley rats. Pairs of rats, one of which had been administered 100 mg/kg probenecid beforehand, were treated with an i.v. bolus dose of 500 mg/kg HMBA. The plasma elimination and urinary excretion of HMBA were then determined. Significant differences (> 25%) in plasma levels or clearance were not observed between untreated and probenecid treated rats as the plasma elimination curves of Figure 1 demonstrate.

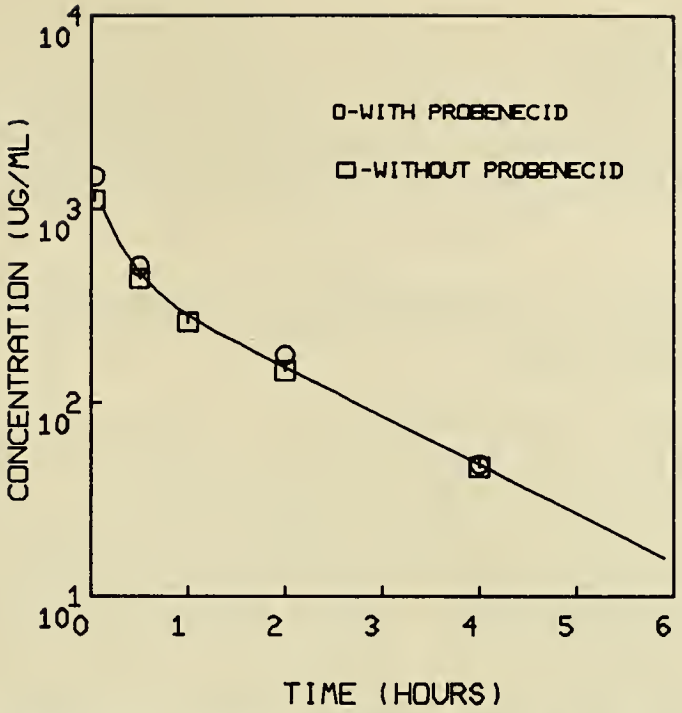
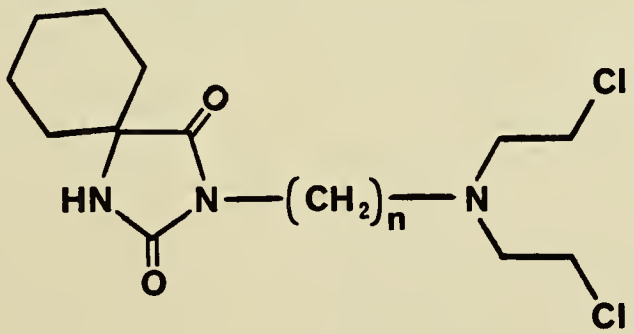


Figure 1. Plasma elimination curves for HMBA given as a rapid infusion of 500 mg/kg in 3 min with (O) and without ([]) probenecid pretreatment.

4. Synthetic and Collaborative Project Support (Dr. Kelley, Ms. Roth):

a. Spirohydantoin Mustard Analogs: The physicochemical characterization of a homologous series of spiropentamethylene hydantoin mustards (7, n = 2-5) was completed in an attempt to relate structure to plasma stability and antitumor activity. Hydrolytic stability correlated with proximity of the hydantoin ring to the nitrogen mustard moiety. This stabilizing interaction of the hydantoin ring was evident in the non-linear increase of GC retention indices as n increased from 2 to 5 and in the lowered pK_a of the mustard nitrogen of spiromustine (n = 2, pK_a = 4.3) as opposed to a pK_a of 6.2 for n = 5.



7

b. Miscellaneous: Numerous samples which cannot be categorized as coming from any one project area were also analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group were Ara-AC and tetrahydrouridine decomposition products, potential acetamide differentiating agents, carboacyclic nucleosides, diazepinone and ring-expanded nucleosides, dideoxyribose nucleosides and neplanocin analogs.

PUBLICATIONS:

1. Litterst, C.L., Roth, J.S. and Kelley, J.A.: Distribution, elimination, metabolism and bioavailability of hexamethylenebisacetamide in rats. Inv. New Drugs, 3: 263-272, 1985.

2. Kelley, J.A., Roth, J.S. and Litterst, C.L.: Gas chromatographic determination of hexamethylene bisacetamide in plasma and urine. Analyt. Lett., 18: 1043-1062, 1985.

3. Curt, G.A., Kelley, J.A., Fine, R.L., Huguenin, P.N., Roth, J.S., Batist, G., Jenkins, J. and Collins, J.M.: A phase I and pharmacokinetic study of dihydro-5-azacytidine (NSC-264880). Cancer Res. 45: 3359-3363, 1985.

4. Huguenin, P.N. and Kelley, J.A.: The synthesis of spiromustine-d_8. A general approach to octadeuterated nitrogen mustards. J. Label. Comp. Radiopharm. 22: 787-797, 1985.

5. Haces, A., Driscoll, J.S., Roth, J.S., Heideman, R.L. and Kelley, J.A.: Spiromustine Analogues. Relationships between structure, plasma stability and antitumor activity. J. Pharm. Sci. 75: 313-316, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM 06177-01 LMC

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

The Analytical Chemistry of Anti-AIDS Agents

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James A. Kelley		Research Chemist	LMC, NCI
Others:	John S. Driscoll	Laboratory Chief	LMC, NCI
	Jeri S. Roth	Chemist	LMC, NCI

COOPERATING UNITS *(if any)*

Laboratory of Pharmacology and Experimental Therapeutics, Laboratory of Experimental Therapeutics and Metabolism, DTP, DCT;
Pediatric Branch, COP, DCT

LAB/BRANCH

Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.0	0.4	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is the research and development of suitable analytical methods to: (1) establish the structure and purity of potential anti-AIDS agents and new antiviral drugs, (2) determine physical and chemical properties of these compounds and their metabolites, and (3) measure these drugs and their metabolites in biological samples to elucidate pharmacology and to determine pharmacokinetics. Gas chromatography, high-performance liquid chromatography and mass spectrometry are emphasized techniques. Compounds of current interest are dideoxycytidine and dideoxyadenosine.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

General Objective:

The objective of this project is the research and development of suitable analytical methods for establishing the structure and purity of new anti-AIDS drug candidates, determining their important chemical and physical properties, elucidating structures of metabolites of these new agents, measuring these compounds and their metabolites in physiological samples and studying reaction mechanisms of synthetically important transformations. Gas chromatography (GC), high-performance liquid chromatography (HPLC) and mass spectrometry are the emphasized techniques. Other analytical methods such as NMR, UV AND IR spectroscopy, and ion exchange and affinity chromatography are also employed.

Specific Objectives:

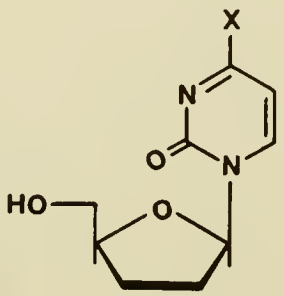
1. Analytical methods development for 2',3'-dideoxycytidine in pharmaceutical and biological matrices.

2. Preclinical pharmacology of dideoxycytidine in mice.

3. Central nervous system pharmacokinetics of dideoxycytidine in non-human primates.

1. Analytical Methods Development for 2',3'-Dideoxycytidine:

a. Rapid HPLC Assay for Dideoxycytidine (Dr. Kelley, Ms. Roth): Dideoxycytidine (1, DDO-C) provides almost complete *in vitro* protection to ATH8 and H9 cells against the infectivity and cytopathic effect of the AIDS virus at drug concentrations of 0.5 - 1 μM. Accordingly, DDO-C is a high priority anti-AIDS clinical candidate. A rapid, simple and sensitive analytical method for DDO-C based on reverse phase HPLC has been developed. This assay is suitable for formulation and for *in vivo* toxicology and pharmacology studies. This assay employs an internal standard (uridine for pharmaceutical solutions; 5-Cl-AraC for biological samples) for accuracy, ultrafiltration for rapid sample preparation, and an isocratic mobile phase (4% CH_3CN/pH 6.8 0.01 phosphate buffer) for simplicity. A high pressure column switching technique is also employed to eliminate interferences from late-eluting plasma components. The limit of quantitation of this assay exceeds 0.2 μM (40 ng/mL) and the within-sample and between-sample coefficient of variation is typically less than 3% for plasma samples spiked at 1 μM (220 ng/mL). Sample isolation and concentration by



1, X = NH_2

2, X = OH

solid phase extraction with a cation exchange cartridge, which offers a four-fold sensitivity increase at the expense of a more complicated analysis, is under evaluation. The use of a Pinkerton internal surface reverse phase HPLC column for the direct analysis of protein-containing biofluids is also being studied. This latter technique may have substantial time and cost advantages for routine therapeutic drug monitoring where a large volume of samples must be analyzed.

b. Solution and Plasma Stability of Dideoxycytidine (Dr. Kelley, Ms. Roth): Oral administration of DDO-C would be a favored means of administering this drug on a chronic basis. Therefore, the stability of 1 has been determined at stomach pH (pH = 1) and 37°. Although some dideoxynucleosides are known to be quite acid labile, the rate of decomposition of DDO-C was quite slow with an 18% loss over 32 hr. Cytosine was a major decomposition product, increasing steadily with time. Less than 5% decomposition was observed over 48 hr for 5 μM DDO-C (1 μg/ml) in pH 7.4 phosphate buffered saline at 37°. Comparable stabilities were observed with the drug in mouse and human plasma at 37°, while monkey plasma showed an approximate 10% decomposition under these conditions. This suggests that DDO-C is not or is a very poor substrate for cytidine deaminase. DDO-C stability will not be a concern in oral administration or in handling biological samples for analysis.

2. Preclinical Pharmacology of Dideoxycytidine in Mice:

a. Bolus Dose Plasma Kinetics (Drs. Kelley, Litterst, Cooney, Ms. Roth): The above HPLC assay was employed to determine the plasma kinetics of 100 mg/kg DDO-C given as an i.v. bolus dose to male BDF_1 mice. Mean plasma levels (n = 4) ranged from 240 μg/mL at 2 min to 120 ng/mL at 6 hr. Plasma elimination of the drug was biphasic with a total body clearance of 30.5 (mL/min)/kg and a terminal phase half-life of 69 min. Urine was the major vehicle for elimination of DDO-C in these mice. Both HPLC and liquid scintillation counting analysis of urine from mice administered ^{3}H-DDO-C indicated that greater than 73% of the 100 mg/kg dose was excreted in 24 hr and more than 94% of this was unchanged drug. A compound corresponding to the monkey metabolite (see Section 3) was not observed.

b. Continuous Infusion Studies (Drs. Kelley, Vistica, Ms. Roth): DDO-C was administered to male BDF_1 mice as a 1-7 day continuous infusion from subcutaneously implanted Alzet mini-osmotic pumps at the rate of 47 (mg/kg)/day. The clearance calculated from the i.v. bolus dose kinetics predicts a steady-state plasma concentration (C_p) of 1.09 μg/mL. Table 1 shows that the two-compartment open model for an i.v. bolus dose reliably predicts plasma levels achievable during continuous infusion.

Table 1. Continuous Infusion of DDO-C in BDF_1 Mice

Infusion Length (days)	Mean Weight (gm)	C_p, Mean ± S.D. (μg/mL)
1	29.2	0.74 ± 0.02
4	29.4	1.12 ± 0.12
7	29.3	1.21 ± 0.45

c. Oral Bioavailability Studies (Drs. Kelley, Johns, Ms. Roth, Mr. Hyman): The bioavilability of an oral dose of 100 mg/kg DDO-C in both fasted and non-fasted male BDF_1 mice was determined. DDO-C was rapidly absorbed from the gut with peak plasma levels being substantially higher (14.1 versus 8.7 µg in fasted mice as in non-fasted animals. In both cases the plasma levels decayed to a 0.5 - 0.8 µg/mL plateau 3-6 hr after drug administration. Estimation of the area under the plasma concentration versus time curve and comparison with that of the i.v. bolus dose study produced an apparent bioavailability of 30% for the fasted mice and 20% for the non-fasted animals.

3. Central Nervous System Pharmacokinetics of DDO-C in Non-Human Primates (Drs. Kelley, Poplack, Johns, Ms. Roth): The cerebrospinal fluid (CSF) and plasma kinetics of a 27 mg/kg (200 mg/m^2) i.v. bolus dose of DDO-C, a dose equivalent to 100 mg/kg in the mouse, have been investigated in male rhesus monkeys with implanted Ommaya reservoirs. These results are listed below in Table 2 and a typical concentration versus time profile is seen in Figure 1. DDO-C rapidly penetrates the CNS to achieve concentrations greater than 1 µM (211 ng/mL); however, CSF levels are much less than corresponding plasma levels and elimination from the CSF parallels plasma elimination.

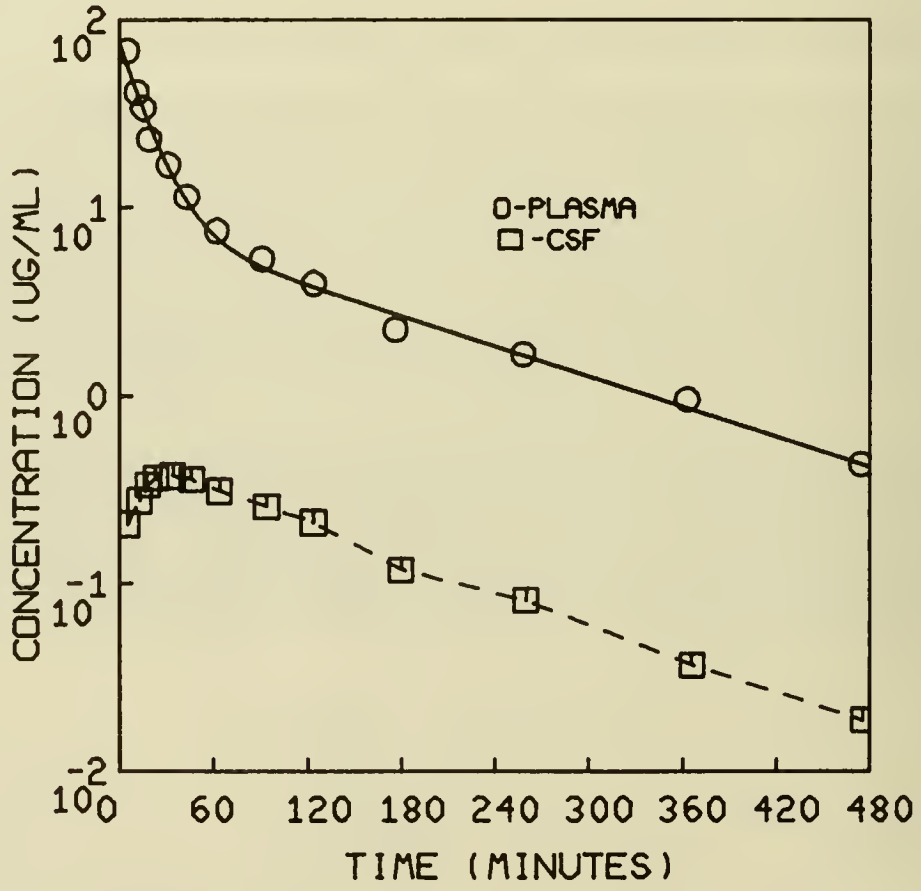


Figure 1. Plasma (O) and CSF ([]) concentration profiles after a single i.v. bolus dose of 27 mg/kg to a 6.1 gk male rhesus monkey (631-T). The plasma elimination curve is the best computer fit (MLAB) to a two-compartment open model while the CSF curve has been drawn point-to-point.

Table 2. Pharmacokinetic parameters for a 27 mg/kg i.v. bolus dose of DDO-C

Monkey	631-T	360-C	102-C
Total dose [mg]	166.4	192	189
^{3}H-DDO-C [μCi]	34.7	40	159.8
Plasma			
Concentration range (μg/mL)	68.7 - 0.43	58.1 - 0.48	51.8 - 0.10
$t_{1/2}(\alpha)$ [min]	11.1	10.9	11.6
$t_{1/2}(\beta)$ [min]	112	102	114
AUC [(μg/mL)-min][a]	2400	2800	2140
Cl_{TB}[(mL/min)/kg]	11.3	9.6	11.8
CSF			
Concentration range [ng/mL]	376 - 19	580 - 22	712 - 45
t_{max} [min]	34	34	23
AUC [mg/mL)/min][b]	65.6	90.4	90.1
CSF/plasma (AUC)[c]	0.026	0.032	0.040

[a] area under the concentration x time curve; calculated from the kinetic model
[b] AUC calculated by trapezoid rule
[c] both AUC's calculated by trapezoid rule with appropriate extrapolation to infinity

Urine was likewise the major vehicle of DDO-C excretion in the monkey. A majority (74%) of the administered radioactivity was excreted in the first 24-hr urine and an average of 50% of the administered dose was present as parent compound. No evidence was found for glucuronide formation, but there was evidence for some minor metabolism. About 5-8% of the sample radioactivity was excreted as 2',3'-dideoxyuridine (2, DDO-U). This metabolite was isolated by semi-preparative HPLC and tentatively identified by NMR and chromatographic comparison with a synthetic sample. Complete structural and biological characterization of this metabolite is in progress. Dideoxyuridine was also observed to appear in both monkey plasma and CSF shortly after DDO-C administration and to reach about the same relative levels seen in urine.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06178-01 LMC

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Applications of New Mass Spectral Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James A. Kelley — Research Chemist — LMC, NCI

COOPERATING UNITS *(if any)*
Laboratory of Chemistry, NHLBI

LAB/BRANCH
Laboratory of Medicinal Chemistry

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objective of this project is research on and development of new mass spectral techniques in order to provide new and/or more rapid solutions to problems involving (1) chemical structure determination, (2) complex mixture analysis and (3) measurement of trace components in biological systems. The utility and scope of these mass spectral methods are determined, and a comparison to other types of analysis, both new and established, is carried out. Fast atom bombardment mass spectrometry and combined liquid chromatography - mass spectrometry are the techniques of current interest. Fast atom bombardment mass spectrometry in both the positive and negative ion mode has been applied extensively to the rapid structure determination of base and sugar-modified nucleosides and synthetic oligonucleotides. A study of spectral anomalies generated by sample-matrix interactions during fast atom bombardment ionization has been initiated. Californium-252 plasma desorption and fast atom bombardment ionization have been compared in the analysis of a series of naturally occurring peroxides.

GPO 914-918

Projection Description:

General Objective:

The objective of this project is the development and application of new mass spectral techniques for the rapid analysis of complex mixtures, measurement of trace components in biological systems and chemical structure determination. Fast atom bombardment mass spectrometry and combined liquid chromatography-mass spectrometry are the new techniques of current interest. The advantages and limitations of these new methods to already established techniques is also an area of concern.

Specific Objectives:

1. Rapid structural analysis of nucleosides and nucleotides by fast atom bombardment mass spectrometry.

2. Spectral anomalies due to matrix-sample interactions during fast atom bombardment mass spectrometry.

3. Comparison of soft-ionization techniques in the analysis of some naturally occurring peroxides.

Major Findings:

1. Rapid Structural Analysis of Nucleosides and Nucleotides by Fast Atom Bombardment (FAB) Mass Spectrometry (Dr. Kelley): The LMC continues to have an extensive program in the synthesis of new nucleosides and nucleotides. Rapid and simple methods employing FAB mass spectrometry have been investigated and uti lized for the characterization of underivatized nucleic acid constituents. Positive ion FAB mass spectrometry provides rapid information on the identity of both the base and sugar in modified nucleosides. Anomers and structures isomeric in either the base or sugar cannot be differentiated, however, since their primary FAB spectra are identical. Negative ion FAB mass spectrometry has been applied to determine the structure and purity of chemically and enzymatically synthesized oligonucleotides. Mass spectral fragment ions provide information on molecular weight, type of phosphate linkage, base modification and sequence. FAB mass spectrometry also allows rapid assessment of nucleotide purity and is useful for analysis of synthetic mixtures containing similar components. Figure 1 indicates that M-H peak heights approximate HPLC peak areas for dinucleotide pyrophosphates. Synthetic artifacts from improper phosphorylation conditions or incomplete removal of protecting groups can also be easily ascertained.

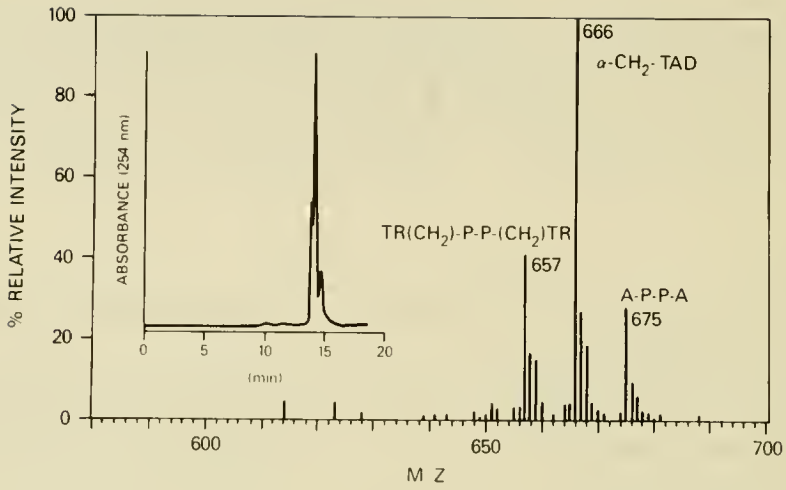
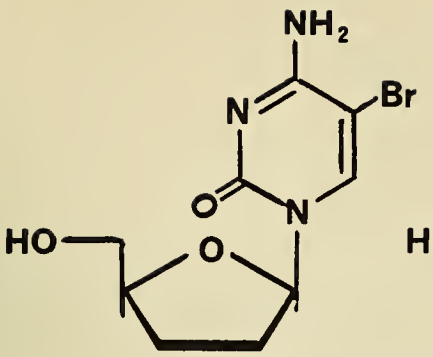
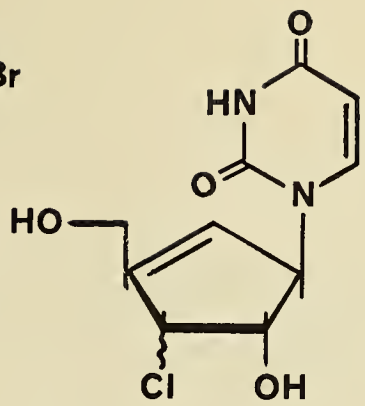


Figure 1. Negative ion FAB spectrum of molecular ion region of synthetic dinucleotide mixture. Insert - HPLC trace.

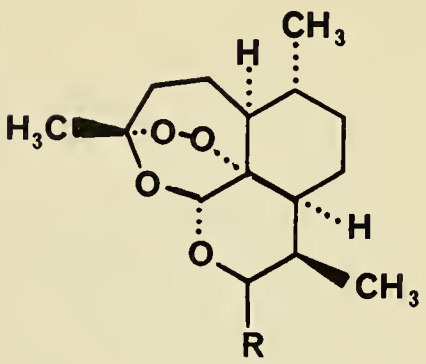
2. Spectral Anomalies Due to Matrix-Sample Interactions in Fast Atom Bombardment (FAB) Mass Spectrometry (Dr. Kelley): Fast atom bombardment mass spectrometry is a newer soft ionization technique that has found wide application in the analysis of thermally-sensitive, polar, high molecular weight materials. Because mass spectra from FAB ionization arise from ion-molecule reactions within the glycerol (or other) matrix, certain complications may be anticipated. The most common of these is that the spectrum of glycerol or matrix, including contaminants in the matrix, is superimposed on the spectrum of the sample. This is no problem if the matrix is well-characterized and chemically stable, since the spectrum of the matrix serves as an internal mass marker and may be computer subtracted. Of more concern is adduct formation due either to the matrix or to ionic contaminants in the sample. These may make molecular weight determination next to impossible by radically changing the spectrum or by completely suppressing ionization of the analyte as in the case of sodium ion contamination. Because sodium is ubiquitous in biological systems, methods for removing this cation such as solid phase extraction are under investigation to improve the quality of FAB spectra of samples isolated from such sources. Halogenated nucleosides are subject to free radical reactions in the FAB liquid matrix. Recognition of these anomalies may aid in the structure determination of these compounds. 5-Bromo-2',3'-dideoxycytidine (1) undergoes free radical debromination in the glycerol matrix to such an extent during FAB analysis that the debrominated nucleoside accounts for the base peak. In contrast, lack of free radical dehalogenation but gas phase elimination of HCl during FAB analysis allowed assignment of structure 2 to a cyclopentenyluridine derivative.



1



2



3a R = O=
3b R = HO

3. Comparison of Soft Ionization Techniques in the Analysis of Some Naturally Occurring Peroxides (Drs. Fales, Kelley). Both californium-252 plasma desorption (PDMS) and fast atom bombardment (FAB) are considered "soft" ionization techniques. PDMS makes use of the sample in the solid phase while in FAB mass spectrometry the analyte is in solution in a liquid matrix. Despite these differences in the mechanics of ion formation, both methods have been reported to give remarkably similar positive and negative ion mass spectra. The positive and negative ion PDMS spectra of 3a and 3b do not give ions clearly indicating the molecular weight of these compounds. The positive and negative ion FAB spectra of these and similar compounds are being examined to determine whether this behavior is general for other forms of particle desorption mass spectrometry.

PUBLICATIONS:

1. Kelley, J.A., Tseng, C.K.H. and Marquez, V.E.: Applications of FAB/MS in the synthesis of NAD analogues and the structure determination of oligoribonucleotides. Adv. Mass Spectrom. (in press), 1986.

2. Marquez, V.E., Tseng, C.K.H., Gebeyehu, G., Cooney, D.A., Ahluwalia, G.S., Kelley, J.A., Dalal, M., Wilson, Y.A. and Johns, D.G.: Thiazole-4-carboxamide adenine dinucleotide (TAD). Analogues stable to phosphodiesterase hydrolysis. J. Med. Chem. (in press), 1986.

ANNUAL REPORT OF THE LABORATORY OF BIOCHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Laboratory of Biochemical Pharmacology was established in January, 1986, by the division of the former Laboratory of Pharmacology and Experimental Therapeutics into two components which are concerned respectively with the design and synthesis of antitumor drugs (Laboratory of Medicinal Chemistry) and the mode of action of new antitumor drugs (Laboratory of Biochemical Pharmacology). The Laboratory studies new agents which have originated within the Developmental Therapeutics Program and also agents derived from extramural sources in whose preclinical development the Program is playing a major role. In the last year, in Laboratory has also participated actively in elucidation of the cellular pharmacology of compounds with anti-HTLV-III activity, currently under development within the Program.

In continuing studies with arabinosyl-5-azacytosine (an antitumor agent developed in this laboratory and now entering clinical trial), mechanisms by which tumor cells become resistant to the drug have been further elucidated. In all the murine systems studied to date, resistance to ara-AC has been accompanied by a fall, and, in some cases, a deletion of deoxycytidine kinase, the enzyme responsible for initiating the anabolic phosphorylation of the drug. In human tumor cell systems, a positive correlation has been established between sensitivity to the drug and deoxycytidine kinase levels. It is proposed that measurement of the kinase may be of prognostic value in patients receiving ara-AC in Phase I/II clinical trials.

2',3'-Dideoxynucleoside analogues of physiological purines and pyrimidines (2',3'dideoxycytidine, adenosine, guanosine and thymidine) are effective inhibitors of the replication of HTLV-III/LAV virus, probably by a viral DNA chain-terminating mechanism. The most potent of these agents, 2',3'-dideoxycytidine, is currently under development for clinical trial. In studies of the cellular pharmacology of this agent in activated human T-lymphocytes in culture (ATH8 cells), we have demonstrated the formation of the mono, di and triphosphates of ddCyd, together with a fourth anabolite tentatively identified as 2',3'-dideoxycytidinediphosphocholine (2',3'-ddCDP-choline). The initial phosphorylation step is catalyzed by deoxycytidine kinase, and does not occur in deoxycytidine kinase-deficient cell lines. The physiological substrate 2'-deoxycytidine will effectively block the antiviral activity of 2',3'-ddCyd by competitively inhibiting anabolism of the analogue. Apart from anabolic phosphorylation, 2',3'-ddCyd is metabolically rather inert; slow deamination (to 2',3'-dideoxyuridine) is detectable in the monkey but not in other species examined to date.

Studies have been initiated on cyclopentenyl-cytosine (CPE-cytosine), an analogue of cytidine in which the ribofuranose has been replaced by a cyclopentenyl ring. This compound, as its triphosphate, is a highly potent inhibitor of CTP synthetase, a key enzyme in de novo pyrimidine synthesis, and it now appears that this property is responsible for its antitumor activity. CPE-CTP has been prepared by enzymatic synthesis and found to have an IC_{50} of 6 μM as an inhibitor of mammalian CTP synthetase.

Studies have continued in the search for new antineoplastic agents directed against tubulin, with emphasis on the detection of compounds which act at sites different from those utilized by known mitotic inhibitors (Vinca alkaloids, maytansine, taxol, colchicine). Compounds currently under study include semi-synthetic analogues of combretastatin, derivatives of 6-benzyl-1,3-benzodioxole, and derivatives of 5,6-diphenylpyridazin-3-one, a mitotic inhibitor which potently stimulates tubulin-dependent GTP hydrolysis, but which binds at a previously undescribed site on tubulin. Parallel biochemical studies have centered on the interaction between the polypeptide subunits of tubulin, its two tightly bound guanine nucleotides, and microtubule-associated proteins (MAPs). Continued progress has been made in the separation of α-tubulin and β-tubulin on a preparative scale and in the purification of microtubule-associated proteins which cause the formation of microtubule bundles and nucleotide interconversions.

Further progress has been made in the exploitation of our earlier observations of qualitative differences between the amino acid transport systems of tumor cells and host bone marrow cells, with tumor cells utilizing System L (the sodium-independent leucine-preferring amino acid transport system) while bone marrow progenitor cells do not. The objective has been the design and synthesis of new amino acid nitrogen mustards which are transported solely by System L and thus exhibit antitumor activity without myleosuppression. The most effective agent to date is 2-amino-7-bis(2-chloroethyl) amino-1,2,3,4-tetrahydronaphthoic acid, the most potent competitive inhibitor of System L (K_i = 0.2μM) described to date, with a 500-fold greater affinity for this transport system than phenylalanine mustard (L-PAM). Comparative evaluation of the antitumor and myelosuppressive activities of L-PAM and the System L - specific nitrogen mustard indicates that the latter possesses both enhanced antitumor and reduced myelosuppressive properties. Synthesis of a larger amount of the naphthoic acid mustard has now been carried out, in order to permit its study in a wider range of experimental tumor systems.

In conjunction with its research output in fields related to the mode of action of new antitumor drugs, the Laboratory of Biochemical Pharmacology continued its active publication record in 1985-86. A total of 27 papers describing these and related studies appeared or were accepted for publication during the current year; these publications are listed in the following section of this report.

Publications:

1. Abraham, I., Dion, R.L., Duanmu, C., Gottesman, M.M. and Hamel, E.: A new antimitotic agent, 2,4-dichlorobenzyl thiocyanate, which alters microtubule morphology. Proc. Natl. Acad. Sci. USA, in press.

2. Ahluwalia, G.S., Cohen, M.B., Kang, G-J., Arnold, S.T., McMahon, J.B., Dalal, M., Wilson, Y.A., Cooney, D.A., Balzarini, J. and Johns, D.G.: Arabinosyl-5-azacytosine II: Mechanisms of native and acquired resistance. Cancer Res., in press.

3. Ahluwalia, G.S., Cooney, D.A., Marquez, V.E., Jayaram, H.N. and Johns, D.G.: Studies on the mechanism of action of tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) VI. Biochemical and pharmacological studies on the degradation of thiazole-4-carboxamide adenine dinucleotide (TAD). Biochem. Pharmacol., in press.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07102-11 LBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Tubulin as a Site for Pharmacologic Attack

PRINCIPAL INVESTIGATOR *(List other professional personnal below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Hamel	Senior Investigator	LBP, NCI
Others:	J.K. Batra	Visiting Fellow	LBP, NCI
	P.S. Chu	Visiting Fellow	LBP, NCI
	C. Duanmu	Visiting Fellow	LBP, NCI

COOPERATING UNITS *(if any)*
Laboratory of Biological Chemistry, DTP, DCT, NCI

LAB/BRANCH
Laboratory of Biochemical Pharmacology

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INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

The goal of this project is the development of new antineoplastic agents directed against tubulin, a protein critical for cell division. Work was initiated with combretastatin congeners, a series of newly isolated natural products, more active than combretastatin itself. The mechanism of action of 2,4-dichlorobenzyl thiocyanate was examined. The drug seems to specifically alkylate the tubulin molecule. Derivatives of 6-benzyl-1,3-benzodioxole continued to interest the laboratory because their facile synthesis permits a structure-function approach to the colchicine/podophyllotoxin binding site of tubulin. Derivatives of 5,6-diphenylpyridazin-3-one, which bind to a distinct site on tubulin, continued to be evaluated in a search for maximally active agents. Alkyl carbamates of aromatic amines were screened in a search for new antitubulin agents. Interactions of tubulin with N-(p-azidobenzoyl)-N'-β-aminoethylvindesine, a photoaffinity analog of vinblastine, were shown to be comparable to those of tubulin with vinblastine.

Microtubules, cellular organelles critical for cell division, are sensitive to a number of antineoplastic drugs. These drugs all cause cells to accumulate in metaphase, disrupting mitosis, for microtubules form the mitotic spindle. The major constituent of microtubules is an acidic protein known as tubulin, and it is the cellular target of virtually all antimitotic drugs. New antimitotic agents continue to be an active area of interest in the laboratory. We are currently studying the following classes of drugs:

1) Analogs of combretastatin. Combretastatin (NSC 348103) is a natural product isolated by G.R. Pettit of Arizona State University from the South African tree Combretum caffrum; and we have demonstrated that combretastatin is a potent inhibitor of tubulin polymerization and binds at the colchicine site of tubulin. Dr. Pettit's group has now purified and characterized a number of additional compounds from Combretum caffrum. We have established that at least three of these agents are considerably more potent than combretastatin itself as tubulin inhibitors, and detailed structure-activity studies are in progress. In addition, several analogs of combretastatin have been synthesized in the laboratory, and their activity is being evaluated.

2) The compound 2,4-dichlorobenzyl thiocyanate (NSC 145813) has been found to inhibit mitosis in murine leukemia cells and, in collaboration with other investigators, to cause clumping or bundling of tubulin-containing structures in Chinese hamster ovary (CHO) cells. In addition, certain lines of CHO cells with mutants in tubulin genes are resistant to NSC 145813. The effects of this agent on in vitro tubulin polymerization are highly unusual. There is minimal change in the reaction unless drug and tubulin are preincubated at 37° (in the absence of GTP to avoid polymerization). With a preincubation low concentrations of the drug completely inhibit tubulin polymerization.

   Radiolabeled NSC 145813 has been prepared and found to bind extremely slowly to tubulin. Moreover, a small portion of the drug binds covalently to tubulin. Polyacrylamide gel electrophoresis of this drug-tubulin complex has demonstrated that both subunits react with the drug, with β-tubulin reacting about twice as extensively as α-tubulin. More remarkably still we have found that tubulin catalyzes an extensive, but relatively slow, breakdown of NSC 145813. The mechanism and products of this reaction are still not established, but its time course correlates better with tubulin inactivation than does the binding of the drug to the protein. No other class of antimitotic drug seems to interfere specifically either with the binding of radiolabeled NSC 145813 to tubulin or with the breakdown of the drug. Structure-activity studies were also initiated with analogs of NSC 145813.

3) A large number of derivatives of 6-benzyl-1,3-benzodioxole have been prepared by Dr. L. Jurd of the Department of Agriculture as potential insect sterilants. A significant number of these compounds have antineoplastic activity and inhibit tubulin polymerization. They are most analogous to podophyllotoxin structurally, and like podophyllotoxin, inhibit both tubulin-dependent GTP hydrolysis and the binding of colchicine to tubulin. Initial

studies established minimal structural requirements for the simplest benzylbenzodioxole derivatives (a 1-3 carbon substituent at position 5, and a methoxy group at position 4' in the benzene ring) which have either an unsubstituted one carbon bridge between the benzene and benzodioxole rings or one or two methyl groups at this bridge position. Additional methoxy substituents on the benzene ring at the 3' and 5' positions, which seemingly increase the structural analogy to podophyllotoxin, resulted in almost complete loss of activity. Recently, several compounds with an ethoxy group at position 4' in the benzene ring have proved active in NCI cancer screens, and we confirmed that these drugs, too, inhibited tubulin polymerization.

Dr. Jurd has also prepared a group of compounds with a third ring (of variable structure) fused to the benzodioxole moiety. The benzene ring is attached directly to this third ring. Several of these agents have potent antitubulin activity, and most of the compounds active against tubulin in vitro also cause mitotic arrest. All compounds have three methoxy groups, attached at positions 3', 4' and 5', on the benzene ring. Although both the third fused ring and the trimethoxy structure appear to substantially increase their analogy to podophyllotoxin, these new agents are more comparable to colchicine in their effects on tubulin-dependent GTP hydrolysis; for, like colchicine, they stimulate rather than inhibit this reaction even while inhibiting the microtubule assembly reaction normally coupled to GTP hydrolysis.

Finally, several additional benzylbenzodioxole derivatives were synthesized in the laboratory. We are currently evaluating them as antitubulin agents.

4) Dr. L. Powers of SDS Biotech Corporation has prepared numerous derivatives of 5,6-diphenylpyridazin-3-one as potential antihypertensive agents. Some of these compounds were found to be potent herbicides as a consequence of inhibition of mitosis in plant tissues. Several members of this class were then submitted to the NCI for screening, and some of these were found to have antineoplastic activity. We have found that a number of these drugs inhibit mitosis in mammalian cells in culture and the polymerization of tubulin in vitro. They potently stimulate tubulin-dependent GTP hydrolysis; but they probably bind at a previously undescribed site on tubulin for they do not inhibit the binding of either colchicine, vinblastine, maytansine or GTP to the protein. Active compounds possess a nitrile group at position 4; and in vitro interactions with tubulin are significantly enhanced by chloride substituents on the phenyl rings, both of which are required for antitubulin activity. There is little overlap between compounds most active against mammalian tubulin and those which are most active in inhibiting mitosis in plant cells. In collaboration with Dr. Powers we are continuing to study structure-activity correlations in this class of drugs to develop maximally active agents.

5) A number of compounds with very different structures have been found to have antineoplastic and antimitotic properties and to inhibit tubulin polymerization. Their only common feature is that they are alkyl carbamates of aromatic amines. A computer search of the NCI drug collection produced over 140 compounds with promising structural features. These were screened

for effects on tubulin-dependent GTP hydrolysis, and over fifty compounds were positive. These in turn were examined for effects on tubulin polymerization, and about a dozen drugs had significant inhibitory activity. These agents are being studied in further detail.

6) Drs. R. Felsted and A. Safa of the LBC, DTP, DCT, have prepared a photoaffinity analog of vinblastine, N-(p-azidobenzoyl)-N'-β-aminoethyl-vindesine. This agent was examined for interactions with tubulin, and its properties are very similar to those of vinblastine. The analog thus appears to be a valid photoaffinity compound for studying the vinca alkaloid binding sites of tubulin.

Publications:

1. Abraham, I., Dion, R.L., Duanmu, C., Gottesman, M.M., and Hamel, E.: A new antimitotic agent, 2,4-dichlorobenzyl thiocyanate, which alters microtubule morphology. Proc. Natl. Acad. Sci. USA, (in press).

2. Batra, J.K., Powers, L.J., Hess, F.D., and Hamel, E.: Derivatives of 5,6-diphenylpyridazin-3-one: synthetic antimitotic agents which interact with plant and mammalian tubulin at a new drug binding site. Cancer Res. 46: 1889-1893, 1986.

3. Batra, J.K., Lin, C.M., Hamel, E., Jurd, L., and Powers, L.J.: New antineoplastic agents with antitubulin activity. Ann. N.Y. Acad. Sci., (in press).

4. Batra, J.K., Jurd, L., and Hamel, E.: Morpholino derivatives of benzylbenzodioxole, a study of structural requirements for drug interactions at the colchicine/podophyllotoxin binding site of tubulin. Biochem. Pharmacol., (in press).

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07104-11 LBP

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*
L-Phenylalanine Mustard Cytotoxicity and Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, leboratory, and institute affiliation)*

PI:	D.T. Vistica	Pharmacologist	LBP, NCI
Others:	S. Ahmad	Visiting Fellow	LBP, NCI
	R. Fuller	Chemist	LMC, NCI
	D. Haines	Visiting Scientist	LMC, NCI
	V. Marquez	Chemist	LMC, NCI
	L. Nguyen	Biologist	LBP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Biochemical Pharmacology

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.4	2.0	0.4

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

L-Phenylalanine mustard (L-PAM) uptake by tumor cells is mediated by two high-affinity amino acid transport systems. The observation that bone marrow progenitor cells either lack System L or exhibit reduced affinity for the System L specific substrate, 2-aminobicyclo [2.2.1] heptane-2-carboxylic acid (BCH) (Blood 56:427) suggested that preparation of new amino acid nitrogen mustards which are transported by System L may result in chemotherapeutic agents which exhibit less myelosuppression. These observations prompted the synthesis of the nitrogen mustard derivative of 2-amino-2-carboxylic-1,2,3,4-tetrahydronaphthalene. Inhibition analysis of the initial rate of transport of BCH by L1210 leukemia cells indicates that this amino acid nitrogen mustard, DL-2-amino-7-bis (2-chloroethyl) amino-1,2,3,4 tetrahydronaphthoic acid, is the most potent competitive inhibitor of System L (K_i=0.2 μM) described to date and has a 500-fold greater affinity for this transport system than phenylalanine mustard. Comparative evaluation of the antitumor and myelosuppressive activities of L-PAM and the System L specific nitrogen mustard indicates that the latter possesses both enhanced antitumor AND reduced myelosuppressive properties. This increased antitumor activity is reflected by a 2-fold improvement in the therapeutic index. In addition to the above mentioned marked differences in the mode of transport and selectivity, this amino acid nitrogen mustard also differs from L-PAM in its rate of dechlorination. These studies indicate a half life ($t_{1/2}$) of 45 minutes as compared to 120 minutes for phenylalanine mustard. This more rapid conversion to a non-cytotoxic derivative may also contribute to reducing host toxicity.

OBJECTIVES:

This project culminates several years of work whose primary objective has been exploitation of a qualitative difference in System L, the sodium-independent leucine-preferring amino acid transport system, in murine L1210 leukemia cells and murine CFU-C. This segment of the project is designed to examine the transport characteristics, antitumor and myelosuppressive activity of DL-2-amino-7-bis(2-chloroethyl) amino-1,2,3,4 tetrahydro-2-naphthoic acid.

METHODS EMPLOYED:

1. CELLULAR TRANSPORT STUDIES: INHIBITION OF THE INITIAL RATE OF TRANSPORT OF 2-AMINOBICYCLO[2.2.1] HEPTANE-2-CARBOXYLIC ACID BY DL-2-AMINO-7-BIS (2-CHLOROETHYL) AMINO-1,2,3,4 TETRAHYDRO-2-NAPHTHOIC ACID.

   Murine L1210 leukemia cells, grown in RPMI 1630 medium containing 16% heat-activated fetal bovine serum, were harvested and washed twice in transport medium composed of, in g/L, $CaCl_2 \cdot 2H_2O$ (0.1), $MgCl_2 \cdot 6H_2O$ (0.1), choline chloride (17.45), HEPES (5.96) and 0.1 mM salt-free bovine serum albumin. The final pH of the transport medium was 7.4. Cells were then incubated with 1.5 µM or 3.0 µM 2Aminobicyclo [2.2.1]heptane2-carboxylic acid [carboxyl ^{14}C] alone or with DL-2-amino-7-bis (2-chloroethyl) amino-1,2,3,4 tetrahydro-2-naphthoic acid. The initial rate of transport of BCH was terminated at 40 seconds by centrifugation of 1 X 10^6 cells thru Versilube F-50 silicone oil. The cell pellets were solubilized in 0.2 N NaOH and counted by liquid scintillation spectrometry.

2. EVALUATION OF THE ANTITUMOR AND MYELOSUPPRESSIVE ACTIVITY OF DL-2-AMINO-7-BIS (2-CHLOROETHYL) AMINO-1,2,3,4 TETRAHYDRO-2-NAPHTHOIC ACID

   Murine L1210 leukemia cells were grown in RPMI 1630 medium containing 16% heat-activated fetal bovine serum. Cells were harvested and washed twice in the same medium. Bone marrow cells were removed from femurs of male CDF_1 mice, washed twice and a cell suspension containing both 100 tumor cells/ml and 100 CFU-C (1.0×10^5 nucleated cells/ml) was prepared. The tumor cells and CFU-C were co-exposed for 45 minutes to DL-2-amino-7-bis(2-chloroethyl) amino-1,2,3,4 tetrahydro-2-naphthoic acid in RPMI 1630 medium containing 16% heat-activated fetal bovine serum. The cells were then harvested, washed twice in McCoy's 5A medium supplemented with 10% fetal bovine serum, 20 units/ml penicillin and 20 µg/ml streptomycin.

Cell survival was assessed following 1 week of growth in the same medium at 37° in a humidified atmosphere of 5% CO_2. Pregnant mouse uterine extract was used as a source of colony stimulating factor for the bone marrow. The presence of either cell type has no effect on the plating efficiency of the other.

MAJOR FINDINGS:

1. The nitrogen mustard derivative of 2-amino-1,2,3,4 tetrahydro-2-napthoic acid is an extremely potent competitive inhibitor of System L. A detailed inhibition analysis of the initial rate of transport of 2-aminobicyclo

[2.2.1] heptane-2-carboxylic acid by DL-2-amino-7-bis (2-chloroethyl) amino-1,2,3,4 tetrahydro-2-naphthoic acid indicates that the latter is the most potent competitive substrate of System L ever described (K_i = 0.22μM). The latter value is ≅ 500 fold lower than that derived for the prototype compound L-phenylalanine mustard (K_i = 110μM) and 25-50 fold lower than that found for 2-aminobicyclo [2.2.1] heptane-2-carboxylic acid.

2. Evaluation of the antitumor and myelosuppressive activity of DL-2amino-7-bis (2-chloroethyl) amino-1,2,3,4 tetrahydro-2-naphthoic acid indicates that the compound possesses both increased antitumor and reduced myelosuppressive activity when compared to its prototype, phenylalanine mustard.

PUBLICATIONS:

1. Ahmad, S., Mulberg, A., Aljian, J. and Vistica, D.T. Hepatic-Mediated Elevation and Maintenance of Metastatic Tumor Cell Glutathione. Biochem Pharmacol., in press.

2. Gram, T.E., Okine, L.K.N. and Gram, R.A. The metabolism of xenobiotics by certain extrahepatic organs and its relation to toxicity. Ann. Rev. Pharmac. Toxicol. 26, 259-291, 1986.

3. Okine, L.K.N., Goochee, J.M., and Gram, T.E. Studies on the distribution and covalent binding of 1,1-dichloroethylene in various tissues of the mouse: Effects of various pretreatments on covalent binding in vivo. Biochem. Pharmacol. 34, 4051-4057 1985.

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5. Okine, L.K.N., Lowe, M.C., Mimnaugh, E.G., Goochee, J.M. and Gram, T.E. Protection by methyprednisolone against butylated hydroxytolune-induced pulmonary damage and impairment of microsomal monooxygenease activities in the mouse: Lack of effect on fibrosis. Exp. Lung Res. 10, 1-22, 1986.

6. Okine, L.K.N. and Gram, T.E. In vitro covalent binding of [^{14}C]1,1-dichloroethylene by kidney, liver and lung of mice. Biochem. Pharmacol., 1986.

7. Okine, L.K.N. and Gram, T.E. Tissue distribution and covalent binding of 1,1dichloroethylene in mice: In vivo and in vitro studies. Proceedings of the Third International Symposium on Biological Reactive Intermediates (BRI III), Plenum Press, New York, 1986.

8. Vistica, D.T., Ahmad, S., Fuller, R. and Hill, J. Transport and Cytotoxicity of Amino Acid Nitrogen Mustards: Implications for the Design of More Selective Antitumor Agents. Federation Proc., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07122-06 LBP

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)*

Biochemical and Pharmacological Studies with Oncolytic Nucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Cooney	Senior Investigator	LBP, NCI
Others:	M. Dalal	Microbiologist	LBP, NCI
	E.G. Gregory	Bio. Lab. Tech.	LBP, NCI
	G. Kang	Visiting Fellow	LBP, NCI
	Y.A. Wilson	Chemist	LBP, NCI

COOPERATING UNITS *(if any)*

Laboratory of Biological Chemistry (R. Glazer and S. Arnold)

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Ara-AC is a hybrid nucleoside combining the structural elements of arabinosyl cytosine and 5-azacytidine. During the past year, systematic studies were carried out to determine the mechanisms by which tumor cells acquire resistance to this agent. Three models were adopted: 1) spontaneous resistance, exemplified by the transplantable colon carcinoma 38; 2) acquired resistance provoked by cultivation of P388 cells in the presence of incremental concentrations of Ara-AC over 100 generations; 3) resistance provoked by mutagenesis with MNNG. In all three cases, the resistance was accompanied by a fall, and in some cases a deletion of deoxycytidine kinase, the enzyme responsible for inaugurating the anabolism of the drug. In the case of the colon carcinoma 38, a second enzymatic factor was identified, namely an abundant endowment with a cytosolic nucleoside triphosphatase, which prevents the accumulation of adequate levels of the proximate antimetabolite, Ara-AC-triphosphate. These studies were next extended to a panel of cultured human tumor cells. In these lines there was a positive correlation ($r = 0.63$) between deoxycytidine kinase levels and the IC_{50} of Ara-AC. It is therefore proposed that measurement of this kinase might well be of prognostic value in patients scheduled to receive Ara-AC in its forthcoming Phase I trials.

Studies with Arabinosyl-5-Azacytosine (Ara-AC)

Ara-AC undergoes extensive anabolism to the 5'-triphosphate which in turn inhibits the synthesis of DNA, but is also incorporated into that macromolecule, where it undergoes ring-opening and engenders inhibition of methylation. During the present year an extensive series of experiments were conducted to determine the mechanism(s) by which tumor cells become resistant to Ara-AC. This study is rendered all the more relevant by virtue of the apparently facile emergence of resistance to Ara-C and 5-AC in patients receiving these agents. Three categories of resistance were examined: spontaneous or native resistance, exemplified by the colon carcinoma 38; resistance provoked by cultivation of P388 cells in the presence of incremental concentrations of Ara-AC over 100 transplant generations; and resistance provoked by exposure to MNNG followed by cloning in agarose. This procedure yielded 8 separate clones, all capable of growing normally in concentrations of the drug ~1000 times those tolerated by the parental strain. Resistance in all of aforementioned cases was found to be associated with a depression in or deletion of the specific activity of deoxycytidine kinase the enzyme which inaugurates the metabolism of Ara-AC. An identical mechanism is very frequently associated with resistance to Ara-C. Interestingly, a second factor contributing to resistance was uncovered in colon carcinoma 38: namely, a rich endowment with a cytosolic nucleotidase, which preferentially degrades nucleoside triphosphates. This activity would of course preclude the maintenance of effective levels of the putative proximate antimetabolite of Ara-AC, namely Ara-AC-triphosphate. Pursuant to these studies with rodent cells and tumors, a prospective examination was conducted of the relationship between the concentration of deoxycytidine kinase and the cytotoxicity of Ara-AC towards a panel of cultured human tumor cells. Although the correlation between these two parameters was positive ($r = 0.633$) it was not perfect. Nevertheless it was clear from the results that tumor cells deficient in deoxycytidine kinase were more refractory to Ara-AC than their well-endowed counterparts.

Studies with cyclopentenyl cytosine (CPE-cytosine).

Cyclopentenyl cytosine is a carbocyclic analog of cytosine in which the ribofuranose moiety has been replaced by a 4-hydroxymethyl-2,3-dihydroxy-4-cyclopentenyl ring. Marked antitumor activity of cyclopentenyl cytosine has been reported in mice bearing L1210 leukemia, P388 leukemia, and B16 melanoma by with its cytocidal activity.

CPE-cytosine is known to be a potent inhibitor of CTP synthesis. We have sought to determine the extent of cyclopentenyl cytosine metabolism, and the possible capacity of any metabolites to inhibit CTP synthetase, a key enzyme in de novo pyrimidine biosynthesis.

Cyclopentenyl cytosine was phosphorylated to its corresponding mono-, di-, and triphosphates in L1210 cells with CPE-CTP being the major metabolite. Partially purified uridine-cytidine kinase catalyzed the phosphorylation of cyclopentenyl cytosine with an apparent Km of 172μM; this is greater than the Km value for cytidine (80μM). Cyclopentenyl cytosine produced a dose dependent decrease in CTP pools. These effects were accompanied by a dose dependent decrease in the specific activity of endogenous CTP synthetase.

Cyclopentenyl cytosine triphosphate from neutralized L1210 cell extracts was separated by HPLC and showed a median inhibitory concentration at 3 μM versus partially purified bovine liver CTP synthetase. Pure cyclopentenyl cytosine triphosphate was synthesized enzymatically and assayed against partially purified bovine liver CTP synthetase to determine the degree of inhibition. The median inhibitory concentration was calculated as 6μM, close to the value obtained using fractions from the neutralized cell extract.

Publications:

1. Ahluwalia, G.S., Cooney, D.A., Marquez, V.E., Jayaram, H.N. and Johns, D.G. Studies on the mechanism of action of tiazofurin (2-β-D-ribofuranosyl-tiazole-4-carboxamide) VI. Biochemical and pharmacological studies on the degradation of thiazole-4-carboxamide adenine dinucleotide (TAD). Biochem. Pharmacol., in press.

2. Ahluwalia, G.S., Cohen, M.B., Kang, G.J., Arnold, S.T., McMahon, J.B., Dalal, M., Wilson, Y.A., Cooney, D.A., Balzarini, J. and Johns, D.G. Arabinosyl-5-azacytosine II: Mechanisms of native and acquired resistance. Cancer Res., in press.

3. Dalal, M., Plowman, J., Breitman, T.R., Schuller, H.M., del Campo, A.A., Vistica, D.T., Driscoll, J.S., Cooney, D.A. and Johns, D.G. Arabinosyl-5-azacytosine I: Antitumor and cytotoxic properties. Cancer Res. 46: 831-838, 1986.

4. Marquez, V.E., Tseng, C.K.H., Gebeyehu, G., Cooney, D.A., Ahluwalia, G.S., Kelley, J.A., Wilson, Y.A. and Johns, D.G. Hydrolytically stable analogs of thiazole adenine dinucleotide (TAD). J. Med. Chem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07166-02 LBP

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Biochemical and Pharmacologic Studies with Oncolytic Barbituric Acid Derivatives

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigetor.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Cooney	Senior Investigator	LBP, NCI
Others:	M. Dalal	Microbiologist	LBP, NCI
	E. B. Gregory	Bio. Lab. Tech.	LBP, NCI
	D. G. Johns	Medical Officer	LBP, NCI
	G. Kang	Visiting Fellow	LBP, NCI
	Y.A. Wilson	Chemist	LBP, NCI

COOPERATING UNITS *(if any)*

J. Covey	Staff Fellow	LMP, NCI
B. Sinha	Cancer Expert	COP, NCI
T. Kensler	Johns Hopkins School of Public Health	

LAB/BRANCH: Laboratory of Biochemical Pharmacology

SECTION:

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on the mechanism of action of merbarone (5-[N-phenylcarboxamido]-2-thiobarbituric acid) have been extended to include the toxicity of this novel oncolytic agent towards human as well as rodent cells. Merbarone is cytocidal to several lung tumor lines propagated in continuous culture, as it is towards murine lymphoblasts, but the median inhibitory concentrations of the drug exhibit a significant spread, ranging from 9 μM in the case of NCI-H358 to 55 μM in the case of NCI-H322. Versus L1210 cells, a strain previously shown to exhibit exquisite sensitivity to merbarone both in vivo and in vitro, the drug produces a powerful time-dependent arrest of DNA synthesis and is accompanied, in human lymphocytes, by chromosomal abnormalities. When L1210 cells are exposed to an IC_{50} concentration of the drug (15 μM) for 16 hours, prominent single strand breaks in their DNA are demonstrable by alkaline elution analyses. These breaks reseal at a rate some fifty times slower than those produced by γ-irradiation. Merbarone also engenders peroxidation of rat liver microsomal lipids in the presence of NADPH and oxygen. On a molar basis this effect is greater than that produced by adriamycin - an oncolytic drug well known for its ability to generate toxic free radicals. However, in contrast to the case with adriamycin, superoxide dismutase did not influence the peroxidation caused by merbarone whereas the antioxidant butylated hydroxyanisole was a potent antagonist of this activity. ESR analyses of microsomes incubated aerobially with merbarone in the presence of NADPH and spin-trapping reagents demonstrated that the drug supported the production of oxygen-centered free radicals and it is hypothesized that these species are responsible both for the DNA strand breaks and lipid peroxidation which result from exposure to merbarone.

In last year's report, the cytotoxic properties of merbarone were outlined in considerable detail. Further studies of this novel agent have been guided by the hypothesis that the drug, in an as yet unknown manner, provokes the generation of oxygen-centered free radicals which damage the DNA of susceptible cells. In favor of this hypothesis is the observation that superoxide dismutase (but not catalase) abolishes the ESR signals seen when merbarone, NADPH and hepatic microsomes are co-incubated. Nevertheless, certain problems beset the full verification of the hypothesis presented earlier. The most salient of these is the failure of merbarone to produce or provoke any oxygen consumption by microsomes (as adjudged by an oxygen electrode) under conditions where adriamycin, a prototype of the class of drugs active by a free-radical mechanism, engenders vigorous oxygen consumption.

This result indicates that there is no net removal of gaseous oxygen from the microsomal systems used in both the ESR and oxygen consumption studies, and suggests that the oxygen-centered radicals provoked by merbarone are short lived, decomposing to regenerate O_2.

One other important consequence of the generation of oxygen-centered free radicals has been documented in the past year in the microsomes used in the studies cited above, marked peroxidation of the membrane lipids (on a molar basis more pronounced than that engendered by adriamycin) is observed as adjudged by the production of thiobarbituric acid-reactive malonaldehyde equivalents. However, paradoxically, superoxide dismutase is without influence on this process, whereas 10 μM levels of the anti-oxidant, butylated hydroxyanisole totally overcome such peroxidation.

Attempts have been made to determine whether superoxide dismutase, butylated hydeoxyanisole and a select number of additional radical or radiation antagonists (WR 2721 and Mesna) could a) overcome the antitumor activity of merbarone *versus* the subcutaneous L1210 tumor (cf summary), b) or nullify the DNA strand breaks caused by this drug in cultured L1210 cells. To date under a variety of experimental conditions, these studies have been negative. These negative results raise the possibility that merbarone cures the L1210 tumor by some mechanism other than, or in addition to, its production of oxygen-centered radicals. As merbarone proceeds through its Phase I trials in man, additional work is needed to establish in an unequivocal way the mechanism(s) by which the drug kills tumor cells *in vivo* as well as *in vitro*.

Publications:

1. Cooney, D.A., Covey, J.M., Kang, G.J., Dalal, M., McMahon, J.B. and Johns, D.G.: Initial mechanistic studies with merbarone (NSC 336628). Biochem. Pharmacol. 34: 3395-3398, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES · PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07179-01 LBP

PERIOD COVERED

October 1, 1985 - September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-protein and Protein-nucleotide Interactions in Microtubule Assembly

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	E. Hamel	Senior Investigator	LBP, NCI
Others:	J.K. Batra	Visiting Fellow	LBP, NCI
	C.M. Lin	Biologist	LBP, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Biochemical Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interaction between the polypeptide subunits of tubulin, its two tightly bound guanine nucleotides, and microtubule-associated proteins. Copolymerization of tubulin·GDP and tubulin·GTP was observed, and reaction conditions which favored incorporation of tubulin·GDP into microtubules were defined. Instability of microtubules to phosphofructokinase and fructose-6-phosphate after exhaustion of GTP in the reaction mixture could not be attributed to breakdown of non-exchangeable GTP bound to tubulin. Continued progress was made in the separation of α-tubulin and β-tubulin on a preparative scale; and in the purification of microtubule-associated proteins which cause the formation of microtubule bundles and nucleotide interconversions.

Microtubules, cellular organelles critical for cell division, are sensitive to a number of antineoplastic drugs. Their major constituent is an acidic protein known as tubulin, which consists of two different polypeptide chains and two molar equivalents of guanine nucleotide. Half this nucleotide (the exchangeable nucleotide) is in the form of either GTP or GDP. If GTP, it is hydrolized to GDP during microtubule assembly from tubulin and microtubule-associated proteins (MAPs -- minor, but essential, components of the microtubule). The remainder of the nucleotide exists only as GTP (the nonexchangeable nucleotide). This GTP is not altered during tubulin polymerization and can only be removed from tubulin by destroying the protein. Its function is unknown.

We have continued our studies on nucleotide interactions at the exchangeable site by defining reaction parameters in which tubulin bearing GDP in the exchangeable site will copolymerize with tubulin bearing GTP. These are all reaction components which increase the relative amounts of tubulin·GDP at the expense of tubulin·GTP--low GTP concentrations if the tubulin concentration is fixed; high tubulin concentrations if the GTP concentration is fixed; GDP in the reaction mixture from any source; low magnesium concentrations. Under conditions most favorable for direct incorporation of GDP into microtubules, up to one-third of exchangeable site GDP can enter microtubules without an initial hydrolysis of GTP. We are currently extending these studies to determine the relative efficiency of incorporation of tubulin·GDP and tubulin·GTP into microtubules; and to determine the minimum ratio of tubulin·GTP to total tubulin required for microtubule assembly to occur. We have also observed that microtubules formed under different reaction conditions differ markedly in their stability properties. We are attempting to determine whether these differences are primarily due to their mechanism of assembly (such as differing amounts of tubulin subunits incorporated with and without GTP hydrolysis) or to their environment (such as the nucleotide composition of the reaction mixture).

We have observed that when phosphofructokinase and fructose-6-phosphate are added to microtubules, even when GTP in the reaction mixture has been exhausted, there is an immediate rapid and extensive depolymerization of the microtubules. Since both the sugar and enzyme are required for this to occur, nucleotide breakdown is implied. Although this in turn suggests that it is the nonexchangeable GTP which is broken down in the disassembly reaction, we have been unable to demonstrate any change in the amount of nonexchangeable GTP bound to tubulin following phosphofructokinase treatment. We are attempting to locate the critical residual GTP required for microtubule integrity.

For many years we have been attempting to reproducibly and preparatively separate the two subunits of tubulin. Although we had achieved significant separation by hydrophobic chromatography, reproducibility has been a problem. Recent experiments have demonstrated that reproducible separation should be feasible in high concentrations of dithiothreitol.

We are continuing to devote a great deal of attention to two classes of MAPs. The first of these causes the formation of microtubule bundles (distinct microtubules which aggregate laterally). The active component appears to be present in MAP preparations in extremely small amounts, although it is highly stable, and has proven more difficult to purify than anticipated. Despite DEAE-cellulose chromatography, ammonium sulfate fractionation, heat-treatment, hyroxyapatite chromatography, and HPLC chromatography (ion-exchange and gel

filtration), the purest preparations remain disappointingly heterogeneous on polyacrylamide gel electrophoresis. We are currently studying whether HPLC hyrophobic or reverse phase chromatography will further improve the protein's purity.

The second class of MAPs of keen interest to us are enzymes which catalyze nucleotide interconversions. This interest began when we observed that extensive ATP formation occurred in microtubule protein preparations if both radiolabeled GTP and adenosine 5'-[β,γ-imido]triphosphate were included in the reaction mixture. Similar results were obtained when either AMP or ADP was substituted for the ATP analog, probably indicating the latter's contamination with other nucleotides. (Repurification of the analog resulted in the disappearance of the nucleotide interconversions observed with the impure compound.) The formation of ATP from AMP + GTP was found to be caused by two closely related nucleoside monophosphate kinases. They copurified on DEAE-cellulose chromatography, ammonium sulfate precipitation, and gel filtration HPLC, but were resolved on hydroxyapatite chromatography. One of the two enzymes was adenylate kinase, as it had maximum activity with adenine nucleotides as both phosphate donor and phosphate acceptor. The other can perhaps most easily be termed "guanylate kinase," for it was able to transfer phosphate groups between guanine nucleotides. This second enzyme was, however, maximally active if the phosphate transfer was between an adenine and guanine nucleotide (either could be donor or acceptor). The formation of ATP from ADP + GTP was caused by nucleoside diphosphate kinase. The enzyme had little nucleotide specificity and was present in abundant amounts in MAPs preparations. It was partially purified by DEAE-cellulose chromatography, ammonium sulfate fractionation, sizing HPLC, hydroxyapatite chromatography, and carboxymethylcellulose chromatography. The nucleoside diphosphate kinase was significantly more important than the nucleoside monophosphate kinase activities in generating ATP from radiolabeled GTP and commercial adenosine 5'-[β,γ-imido]-triphosphate.

Publications:

1. Duanmu, C., Lin, C.M., and Hamel, E.: Tubulin polymerization with ATP is mediated through the exchangeable GTP site. Biochim. Biophys. Acta 881: 113-123, 1986.

2. Hamel, E., Batra, J.K., Huang, A.B., and Lin, C.M.: Effects of pH on tubulin-nucleotide interactions. Arch. Biochem. Biophys. 245: 316-330, 1986.

3. Hamel, E., and Lin, C.M.: Dideoxyguanosine nucleotides and microtubule assembly. Ann. N.Y. Acad. Sci. (in press).

4. Huang, A.B., Lin, C.M., and Hamel, E.: Maytansine inhibits nucleotide binding at the exchangeable site of tubulin. Biochem. Biophys. Res. Commun. 128: 1239-1246, 1985.

5. Huang, A.B., Lin, C.M., and Hamel, E.: Differential effects of magnesium on tubulin-nucleotide interactions. Biochim. Biophys. Acta 832: 22-32, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07180-01 LBP

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*: Hepatic-Mediated Resistance of Tumor Cells to Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.T. Vistica	Pharmacologist	LBP, NCI
Others:	S. Ahmad	Visiting Fellow	LBP, NCI

COOPERATING UNITS *(if any)*:

LAB/BRANCH: Laboratory of Biochemical Pharmacology

SECTION:

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.0	0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Metastatic migration of the murine L1210 leukemia and an L-phenylalanine mustard resistant variant (L1210/L-PAM) from the peritoneal cavity and infiltration into the liver results in a 2-fold increase in the glutathione (GSH) content of both tumor cells. These increases in the cellular content of GSH are accompanied by a 2-fold increase in the resistance of these tumor cells to L-phenylalanine mustard. Cell surface labeling studies indicate that cells isolated from the liver have a 5-fold greater content of surface sulfhydryl groups as compared to their ascitic counterparts. The former can be sensitized to L-phenylalanine mustard by blocking these sulfhydryl groups by continuous exposure of cells to 6-6' dithiodinicotinic acid.

Studies utilizing co-cultures of murine hepatocytes and murine leukemia cells in serum-free medium indicate an absolute requirement for the hepatocyte in the promotion of tumor cell growth.

These results demonstrate a determinant role of the hepatic microenvironment in tumor cell growth, maintenance of tumor cell GSH, cell surface sulfhydryl groups and the sensitivity of these tumor cells to L-phenylalanine mustard.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

This project is designed to examine the role of the environment of the host organ on tumor cell proliferation and sensitivity to chemotherapeutic regimens.

Methods Employed:

A. Maintenance of Tumors

The murine L1210 leukemia and a line developed for resistance to L-phenylalanine mustard (L1210/L-PAM) were maintained in male CDF_1 mice by weekly intraperitoneal injection of 1 x 10^5 and 1 x 10^6 cells respectively.

B. Isolation of Tumor Cells from the Ascites and Liver

Animals bearing 7 day tumors were anesthesized with 5% chloral hydrate. Tumor cells were removed from the peritoneal cavity and used for determination of cellular glutathione and sensitivity to L-phenylalanine mustard as described below.

Tumor cells were isolated from the liver following sequential perfusion of calcium and magnesium free Hank's balanced salt solution containing 1mm EGTA followed by 100 units/ml collagenase in William's Medium E. The liver was removed and teased apart in Dulbecco's phosphate buffered saline (without calcium and magnesium) containing 0.1mm sulfhydryl modified bovine serum albumin and 10mm glucose. The cell mixture, containing tumor cells and hepatocytes, was centrifuged at 300 x g and the cells washed twice in Dulbecco's phosphate buffered saline containing 0.1mm sulfhydryl modified bovine serum albumin and 10mm glucose. The cells were then resuspended in 50ml of the same buffer system in a 50ml Falcon conical centrifuge tube and allowed to stand undisturbed at 25°C for 60 minutes. The top 30ml containing tumor cells was removed and utilized for determination of cellular glutathione and sensitivity to L-phenylalanine mustard as described below.

C. Isolation of Hepatocytes

Non-tumor bearing mice were perfused according to the procedure described above. Hepatocytes were centrifuged at 60 x g for 5 minutes, washed in William's Medium E containing 1% fetal calf serum and allowed to attach to 35mm petri dishes coated with fibronectin and collagen. The unattached, non-viable cells were removed at 30-45 minutes and fresh medium added. Tumor cells from the peritoneal cavity of mice were inoculated into the plates 24 hr. later and tumor cell growth monitored.

D. Determination of Cellular Glutathione Content

Tumor cells from the ascites and liver ($2x10^7$) were washed twice in Dulbecco's phosphate buffered saline containing 0.1mm bovine serum albumin and red blood cells removed by hypertonic lysis in 0.87% ammonium chloride for 5 minutes at room temperature. Cells were then washed once in Dulbecco's Phosphate buffered saline, pelleted by centrifugation at 300 x g for 10 minutes and lysed in distilled water. Sulfosalicylic acid was added to a final concentration of 3% and protein removed by centrifugation at 12,000 x g for 2 min. Glutathione (GSH) and its disulfide (GSSG) were assayed by the standard spectrophometric assay of Griffith.

E. Evaluation of L-phenylalanine Mustard Cytotoxicity

Cells, isolated from the ascites and liver, as described above, were suspended in RPMI 1630 medium containing 16% heat-inactivated fetal calf serum, 40 μg/ml gentamicin and 50 μM B-meraptoethanol. Cells were exposed to L-phenylalanine mustard for 2 days and surviving cells estimated by clonal growth in soft-nutrient agar for 2 weeks.

F. Determination of Membrane Sulfhydryl Content of Cells Isolated from the Ascites and Liver

Cells in Dulbecco's phosphate buffered saline containing 0.1mM sulfhydryl modified bovine serum albumin were exposed to 100 μM [^{14}C] 6,6'-dithiodinicotinic acid for 15 minutes at 37°C. Cells were washed twice in the same buffer system and treated with 1mm glutathione to release the bound radiolabel. Cells were then removed by centriugation thru versilube F-50 silicone oil and the supernatant was was counted by liquid scintillation spectrometry.

Major Findings:

1. Metastatic migration from the peritoneal cavity and infiltration into the liver results in a 2-fold increase in the GSH content of both L1210 and L1210/L-PAM tumor cells.

2. These increases in the cellular content of GSH are accompanied by a 2-fold increase in the resistance of these tumor cells to L-phenylalanine mustard.

3. These observed differences in GSH content and sensitivity to L-phenylalanine mustard in cells isolated from the ascites and liver are observed only *in vivo* since short term culture results in cells with similar GSH content and sensitivity to L-phenylalanine mustard.

4. Cell surface labeling studies indicate that cells isolated from the liver have a 5-fold greater content of surface sulfhydryl groups as compared to their ascitic counterparts. The former can be sensitized to L-phenylalanine mustard by blocking these sulfhydryl groups with 6-6' dithiodinicotinic acid.

5. Studies utilizing co-cultures of murine hepatocytes and murine leukemia cells, in culture medium devoid of serum, indicated an absolute requirement of the hepatocyte for growth of tumor cells.

6. These results demonstrate a determinant role of the hepatic microenvironment in the promotion of tumor cell growth, in the maintenance of tumor cell GSH, cell surface sulfhydryl groups and the sensitivity of these tumor cells to L-phenylalanine mustard.

Publications:

1. Ahmad, S., Mulberg, A., Aljian, J. and Vistica, D.T. Hepatic-Mediated Elevation and Maintenance of Metastatic Tumor Cell Glutathione. Biochem. Pharmac., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-07181-01 LBP

PERIOD COVERED: October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Biologic and Pharmacologic Studies with Antiviral Nucleosides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	D.A. Cooney	Senior Investigator	LBP, NCI
Others:	M. Dalal	Microbiologist	LBP, NCI
	E.B. Gregory	Bio. Lab. Tech	LBP, NCI
	G. Kang	Visiting Fellow	LBP, NCI
	D.G. Johns	Medical Officer	LBP, NCI
	V. Marquez	Visiting Fellow	LMC, NCI
	Y.A. Wilson	Chemist	LBP, NCI

COOPERATING UNITS *(if any)*

Dr. Jan Balzarini, Clinical Oncology Program, NCI
Dr. H. Mitsuya, Clinical Oncology Program, NCI

LAB/BRANCH: Laboratory of Biochemical Pharmacology

SECTION:

INSTITUTE AND LOCATION: NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	1.0	1.5

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☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standerd unreduced type. Do not exceed the space provided.)*

In human lymphocytes, the antiviral nucleoside 2',3'-dideoxycytidine undergoes substantial anabolism but negligible catabolism. Using tritiated drug and authentic standards as markers, the mono, di and triphosphate forms of the compound have been identified in human, murine and caprine cells exposed to 1μM nucleoside for twenty-four hours. In ATH8 cells, the concentration of dideoxycytidine-5-triphosphate reached under these conditions approximated 0.5μM, a level capable of inhibiting retroviral reverse transcriptase, presumably by a chain-terminating mechanism. A fourth metabolite with unique chromatographic behavior was also identified. Because this metabolite resisted attack by alkaline phosphatase but was decomposed by venom phosphodiesterase, it was concluded to be a phosphodiester; because it incorporated label from [^{14}C] choline as well as from [^{3}H] dideoxycytidine, it is postulated to be dideoxycytidine diphosphocholine. In studies of the catabolism of dideoxycytidine, no deamination of the nucleoside nor of its 5'-monophosphate could be demostrated with murine enzymes; however, activities contaminating bacterial phosphorylases did slowly deaminate the drug.

PHS 6040 (Rev. 1/84) GPO 914-918

When 2'3'-deoxynucleosides are incorporated into DNA, they function as efficient chain-terminators. Retroviral reverse transcriptase is especially susceptible to this mode of inhibition. Dr. S. Broder and his colleagues at NCI have demonstrated that many 2'3'-dideoxynucleosides are effective in inhibiting the replication of the HTLVIII/LAV virus using infected human lymphocytes in culture (ATH8 cells) as the host - but that dideoxycytidine is especially potent in this system, its IC_{50} being ~ 0.5μM. On the basis of this activity, the AIDS Drug Development Committee voted to develop dideoxycytidine for clinical trials. Preparatory to these trials, we have begun to examine certain of the pharmacologic properties of this nucleoside in human and murine cells. In the several lines examined to date, tritiated dideoxycytidine is taken up rapidly, a process which is inhibited strongly by nitrobenzylthioinosine, a prototypical inhibitor of the faciliated diffusion of many nucleosides, but only modestly (50%) by a 50-fold molar excess of its natural congener, 2'deoxycytidine. Once interiorized, dideoxycytidine is anabolized to at least 4 nucleotide species: its 5'-mono, di, and triphosphates, and a novel phosphodiester believed to be dideoxycytidine diphosphocholine on the basis of studies with radiolabeled precursors. The concentration of the 5'-triphosphate-which is presumed to function as the proximate chain-terminating species-varies widely from cell-line to cell-line following 24 hr of incubation with 1μM drug, but in ATH-8 lymphocytes this nucleotide reaches levels of ~ 0.5μM, a concentration capable of inhibiting reverse transcriptase under assay conditions in which dCTP is limiting (endogenous reaction).

Studies were next undertaken on the influence of deoxycytidine on the production of these metabolites. A 1μM concentration of this natural nucleoside reduced all metabolites of dideoxycytidine by 70%; a 10-fold molar excess (10μM) totally blocked anabolism of the drug. In analogous experiments, the biotransformation of dideoxycytidine was examined in a line of murine macrophage/lymphoblasts devoid of deoxycytidine kinase: these cells carried out little or no phosphorylation of dideoxycytidine, a result which supports the concept that deoxycytidine kinase inaugurates the metabolism of both nucleosides.

Catabolism

A partially purified preparation of cytidine deaminase from mouse kidney failed to deaminate dideoxycytidine even after protracted incubations. This result is in keeping with the empirical rule that this hydrolytic enzyme requires a 3'hydroxyl in the "down" position for activity to be manifest. Similarly, crude deoxycytidylate deaminase failed to attack chemically-synthesized dideoxycytidine-5'-monophosphate under experimental conditions in which deoxycytidylate was quantitatively deaminated. These results make it unlikely that deamination is an important metabolic fate of this compound. By contrast, it has been observed that several bacterial enzymes (uridine and thymidine phosphorylases, from E. coli), probably as a consequence of contaminating activities, do catalyze slow deamination of dideoxycytidine, but the quantitative importance of this activity remains to be assessed. In this connection, it is important to point out that dideoxyuridine is therapeutively inactive versus HTLV-III (data of Jan Balzarini and Samuel Broder).

Publications:

1. Cooney, D.A., Dalal, M., Mitsuya, H., McMahon, J.B., Nadkarni, M., Balzarini, J., Broder, S. and Johns, D.G. Initial studies on the cellular pharmacology of 2',3'-dideoxcytidine, an inhibitor of HTLV-III infectivity. Biochem. Pharmacol., (in press).

ANNUAL REPORT OF THE LABORATORY OF MOLECULAR PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The Laboratory of Molecular Pharmacology during the current year has carried out studies in 4 major areas: (1) studies of the molecular mechanisms of action of alkylating agents, including chloroethylnitrosourea-like compounds and nitrogen mustards; (2) studies of topoisomerase II as a target of action of DNA intercalating agents and epipodophyllotoxins; (3) studies of the effects of incorporation of base analogs into cell DNA on DNA structural integrity as measured by DNA filter elution teqhniques; (4) studies of the regulation of chromosomal protein biosynthesis in relation to the control of cell proliferation. A major goal is to develop strategies for the selective killing of particular human tumor cell types.

DNA Chloroethylating Agents

Previous work in the LMP has shown that the DNA repair protein, guanine-O6-alkyltransferase (GO6AT), affects the sensitivity of human tumor cell strains to chloroethylating agents, such as chloroethylnitrosoureas, clomesone and mitozolomide. About 25% of human tumor cell strains that have been tested are deficient in GO6AT, and these cell strains exhibit increased sensitivity to killing by chloroethylating agents, compared to cell strains having normal GO6AT levels. The mechanism of this phenomenon has been elucidated by the work of several laboratories, including the LMP. The drugs link a chloroethyl group onto guanine-O6 positions in DNA. These chloroethyl groups are rapidly removed by GO6AT, if GO6AT is present at adequate levels in the cell. In cells that are deficient in GO6AT, the O6-chloroethylguanines in DNA can react further to form possibly lethal interstrand crosslinks.

These findings suggest that a tumor in a given patient may respond to treatment with a chloroethylating agent only if the tumor has the GO6AT-deficient (Mer^-) phenotype. We are therefore developing a simple and sensitive assay for GO6AT.

The assay employs a synthetic double-stranded deoxyoligonucleotide which was designed so as to incorporate an O6-methylguanine residue in a sequence recognized by a restriction enzyme. While the methyl group remains in place, the restriction enzyme does not recognize the site and does not cleave the site. When the oligonucleotide is incubated with a cell or tissue extract that contains GO6AT activity, some of the methyl groups are removed, generating sites that the restriction enzyme can cleave. Cleaved strands (which bear a a ^{32}P label at one end) are currently detected by a gel electrophoretic method that we have developed to allow the loading of relatively large samples. In order to simplify the assay further, the oligonucleotide will be attached to a solid support which will permit a very easy separation and assay of the cleaved fragment. The present assay has successfully identified Mer^+ and Mer^+ phenotypes in human tumor cell strains and in xenograft tumors.

DNA Base Sequence Selectivity of Alkylation by Nitrogen Mustards

The selectivity of nitrogen mustards for reaction with guanines in DNA and the frequent occurrence of G-rich regions in oncogenes suggested to us that DNA base sequence selectivity may play a role in the anti-tumor activity of these drugs. If this is true, then the effectiveness of these drugs might be substantially improved by the design of compounds that would optimize the base sequence selectivity. We are therefore studying the DNA base sequence selectivity of reaction of various nitrogen mustards, in order to achieve a structural understanding of the origin of this selectivity.

During the past year we have constructed a set of computer programs that process data obtained by densitometric scanning of autoradiograms of DNA sequencing gels. The programs compute corrected reaction intensities for individual bases in the sequence, and do so almost completely automatically and objectively. Using this method of analysis we have confirmed our previous inferences about the role of base-sequence-dependent electrostatic potential at guanine-N7 positions in governing the reaction intensities of nitrogen mustards such as HN2, melphalan, spiromustine and phosphoramide mustard. We find also that these electrostatic effects are reduced by increasing the salt concentration of the solvent. Since long-range electrostatic effects in chromatin are likely to be diminished by the presence of cationic molecules, other sources of selectivity would be more relevant biologically. We have identified 2 examples of selectivity not attributable to electrostatic potential. These appear in the reactions of uracil mustard and quinacrine mustard. In both cases, the sequence selectivity data have allowed us to formulate structural hypotheses which we will test by molecular graphics modelling.

DNA Topoisomerases as Targets of Anticancer Drugs

Previous work in this Laboratory, using DNA filter elution techniques developed here, had shown that several DNA intercalating anticancer drugs (e.g. amsacrine, anthracyclines and ellipticines) produce DNA strand breaks and DNA-protein crosslinks in mammalian cells. The pattern of these effects indicated that DNA strands are cleaved and that at the same time a protein becomes covalently linked to the DNA at the cleavage sites. We refer to this type of DNA lesion as protein-associated strand break (PASB). We proposed that these effects are due to drug action on one or more DNA topoisomerases. Work in several laboratories has confirmed the observations and has extended the list of drugs that produce PASB. In addition to new intercalating agents, such as mitoxatrone, the list of drugs that affect topoisomerases now includes the epipodophyllotoxins and camptothecin.

Our inferences of drug actions on topoisomerases were confirmed upon isolation and characterization of mammalian topoisomerase II by Liu and coworkers who found that the purified enzyme responds to drugs in the manner predicted by the DNA filter elution findings in intact cells. Concurrently, we isolated the factor that produces drug-induced PASB in intact cells. During the past year, we completed the identification of this factor as topoisomerase II. As a result of this work, there is now a firm foundation for the use of DNA filter elution assays for the determination of topoisomerase alterations in intact cells.

We are currently studying the topoisomerases in a drug-resistant cell line that exhibits a markedly reduced PASB response to amsacrine. The reduced PASB response is demonstrable in isolated cell nuclei as well as in intact cells and therefore is not attributable to limited drug uptake. We have found unexpectedly that the topoisomerase II isolated from the resistant cells has normal drug response characteristics and that the amount of enzyme per cell is only moderately reduced. However a new topoisomerase activity was fractionated from the resistant cells that was not detected in the sensitive parental cells. This possibly new topoisomerase is now under study.

We have found that topoisomerase II (measured by drug-induced PASB) undergoes changes during the cell cycle. Quiescent 3T3 cells were stimulated to proliferase and topoisomerase II responses to amsacrine or etoposide were examined at various times during the first cell cycle. A large wave of increased response was noted approximately during the S-phase period. Since increased PASB responses have recently also been reported in mitotic cells, we plan to isolate and compare the topoisomerases that are enhanced under different conditions.

Effects of Incorporation of Base Analogs into DNA

Although the DNA filter elution technique has been used to study the effects of many agents that damage DNA by various mechanisms, there has been very litte previous study of the effects of the incorporation of base analogs into DNA. We have now completed studies of the effects of incorporation of 3 base analogs: 5-aza-2'-deoxycytidine (aza-dC), 5,6-dihydro-5-azacytidine (H_2-aza-C) and 6-thioguanine (tG). Interesting and unexpected results were obtained in each case.

Aza-dC was found to produce alkali-labile sites in those DNA strands that were synthesized during exposure of cells to the drug. DNA strands synthesized prior to drug exposure did not exhibit these lesions. The alkal-labile sites persisted with no detectable decline in 24 hrs. The findings are consistant with the known chemical instability of aza-dC. We hypothesize that azacytosine residues in DNA are alkali-labile and generate strand breaks by a chemical reaction mechanism analogous to that which generates strand breaks from base-free sites in alkali. The results indicate that the DNA filter elution technique can be used to determine the frequency of incorporated azacytosine residues and to separate genomic regions containing high frequencies of these residues.

H_2-aza-C was originally designed as a possible chemically stable pro-drug of aza-C. We found however that H_2-aza-C does not generate detectable alkali-labile sites in L1210 cells. Hence H_2-aza-C does not lead to the incorporation of azacytosine residues (at least in L1210 cells), and is not a prodrug for aza-C in these cells. Moreover, H_2-aza-C produced effects not produced by aza-dC: (1) DNA strand breaks not of the alkali-labile type, possibly generated in the course of DNA excision repair, and (2) a striking and unusualy inhibition of elongation of replicating DNA strands. Thus H^2-aza-C was shown to be a distinct drug having effects unrelated to those produced by aza-dC.

Thioguanine (tG) was previously reported to produce DNA strand breaks. Our studies confirmed the production of a low frequency of strand breaks, but

more prominently we observed a previously unreported formation of DNA-protein crosslinks. Although the DNA-protein crosslinks were most numerous in DNA strands that were synthesized while the cells were exposed to drugs, we were able to demonstrate a crossover of the DNA-protein linking effect to DNA strands whose synthesis was completed prior to the addition of drug. This unexpected result suggests that tG incorporated in one DNA strand can become linked to a protein (perhaps through disulfide linkage) that topologically encompases both complimentary DNA strands even after treatment with detergent and alkali. It would be of considerable interest to isolate and identify this linked protein.

Regulation of Chromosomal Protein Biosynhtesis in Relation to the Regulation of the Cell Cycle

We had recently found that proliferating cells can compensate for imposed reductions in protein synthesis by selectively increasing the rates of synthesis of histone proteins that are needed for the assembly of S-phase chromatin. This compensation mechanism allows cells to complete DNA replication and mitosis in a nearly normal time period despite the partial inhibition of over-all protein synthesis by low concentrations of drugs such as cycloheximide. Studies during the current year showed that the compensation mechanism involves specific increases in histone m-RNA levels. Studies are in progress to determine the generality of the histone compensation response following various means of limiting protein synthesis. The histone compensation response is also being examined in various transformed cell types in order to determine whether transformed or neoplastic cells may be defective in this regard.

In order to investigate further the mechanisms that regulate histone syntheses one must be able to measure the quantity of various histones in the cytoplasmic pool. New methodology however had to be developed to allow measurement of the small quantitites of histones present in the pools. The development of such methods was successfully completed during the past year. Studies are in progress to determine changes in histone pools during the cell cycle and in response to drugs.

Previous work in this Laboratory had disclosed differences between the set of histone variant proteins that are synthesized in quiescent (G0) cells, G1 cells and S phase cells. Work completed during the current year investigated effects of various DNA synthesis inhibitors and DNA damaging anti-neoplastic agents. A variety of effects were observed on histone variant synhtesis patterns and a model to explain these effects was proposed.

Work is under way to isolate a gene for a histone variant protein (H2A.Z) that is regulated characteristically in G0 cells. Analysis of the DNA sequences that regulate the transcription of this gene may help to elucidate the control mechanism for cell proliferation at the genomic level. A number of clones from a human genomic library in charon 28 lambda were selected on the basis of hybridization to two oligonucleotide probe sets which were synthesized based on the amino acid sequence of the unique portion of the H2A.Z protein

In order to proceed further, it was necessary to test the candidate clones against mRNA species that are present in cells at very low levels.

This required a new method mRNA isolation, which we have now developed. The new method allows the simple and rapid isolation of small quantities of RNA from whole cells or cytoplasmic extracts. The method should find wide application in molecular cell biology.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06140-10 LMPH

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Regulation of Histone Biosynthesis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objective is to elucidate the mechanisms regulating histone biosynthesis in G1, S and G0.

Using methodology developed in our group over the last several years to resolve and characterize histone variants, we have been analyzing the patterns of histone synthesis during different cell behavioral states. Histones were found to be synthesized not only during S-phase, but also during G1 and the quiescent state (also termed extended G1 or G0). The qualitative pattern of histone synthesis differs between S-phase, G1 and quiescent cells, a finding which shows that the synthesis in G1 or quiescent cells is not due to contamination by S-phase cells. The histone synthesis in both G1 and quiescent cells is not linked to DNA synthesis. Histones synthesized in quiescent cells are stable and seem to be incorporated into chromatin. The results suggest that the quiescent state is not an extended G1 phase but a discrete state. This year, we published a comprehensive model concerning the regulation of histone and DNA synthesis. The cytoplasmic histone pool is a crucial factor in this model and methods for analyzing the histone pool are being worked out.

There is some evidence in the literature which suggests that there may be significant differences in the regulation of chromatin biosynthesis between normal and tumorigenic cells. Understanding these differences may lead to the development of protocols to protect normal cells or make tumorgenic cells more vulnerable to various treatments.

Project Description

Introduction:

We have found that when dividing cells cease division and become quiescent, they continue to synthesize histones at a reduced but significant rate. The pattern of synthesis of variant histones in quiescent cells differs from that in S-phase; therefore, this synthesis cannot be attributed to the presence of S-phase cells in the culture.

In CHO cells where the pattern of histone synthesis has been examined during the cell cycle, reduced but significant synthesis has been found in both G2 and G1 (Wu and Bonner, Cell 27: 321-330, 1981). This basal pattern differs from both the S-phase and the quiescent patterns. These results strongly suggest that the quiescent state is not merely an extended G1, but is a discrete state or cycle.

Objectives:

1) The development of greater understanding of the molecular mechanisms regulating chromatin biosynthesis and metabolism during the cellular states of proliferation and nonproliferation.

2) The characterization of chromatin or cellular components that may be involved in these regulatory mechanisms.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional eletrophoresis. (Methods developed in this laboratory).

(2) Peptide analyses on acrylamide gels to determine the relationship of proteins to each other. (Method developed in this laboratory).

(3) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(4) Maintenance of cells and nuclei in viable non dividing states using modified and defined media.

(5) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells. Cell free translation of mRNA.

Major Findings and Accomplishments:

1. Histone H1 Biosynthesis in Various Cell States

We have extended our studies on the patterns of histone synthesis during different physiological states to induce the fifth class of histones, H1.

The pattern of H1 variant synthesis is very different between growing and quiescent cells. The synthesis of the variants H1.1 and H1.2 was negligible

in quiescent IMR-90, but significant when these cells were proliferating. In resting lymphocytes only 3 variants appear to be synthesized, namely H1.3, H1.4 and H1.5, but after 50 hours of stimulation with PHA another subtype, likely to be H1.2 is synthesized in significant amounts. In IMR-90 cells treated with hydroxyurea (HU) and 10 μM cytosine arabinoside (Ara-C), the H1 synthesis pattern was found to be similar to that observed in quiescent cells. The results of this study have been published.

2. Effects of Inhibitors of DNA and Protein Synthesis on G1, Quiescent, and S-Phase Histone Synthesis to DNA Synthesis

Many studies have shown that inhibition of DNA synthesis immediately leads to a similar inhibition of histone synthesis even though total protein synthesis is not significantly inhibited. Our studies with hydroxyurea, a classical inhibitor of DNA synthesis, show that basal and S phase histone synthesis are inhibited to different extents when DNA synthesis is inhibited. We have continued these studies with a variety of inhibitors of DNA synthesis. Those agents which inhibit DNA synthesis only, give the same results as hydroxyurea. However other agents which also inhibit protein syntheses do not selectively inhibit the synthesis of particular histone variants but change the histone synthesis pattern in other ways. The crosslinking antineoplastic agents are in this class. It has previously been reported that inhibition of protein synthesis blocks the specific effect of DNA synthesis inhibitors on histone synthesis. We have published a model that offers an explanation for these interactions.

3. Development of Methodology to Study Histone Pools

The amount of histone in the cytoplasm is thought to be a central control element in the regulation of histone and DNA synthesis. However, the histone pool has not been studied in any systematic way because of several technical difficulties. These include the small size of the pool, the problems of purifying histone proteins from the cytoplasm, and the problem of distinguishing cytoplasmic histone from contaminating chromatin histone. Those few studies of histone pools have examined the labeling kinetics of some of the histone proteins rather than their mass.

We have adapted our methodology for the analysis of histone variants in chromatin to the analysis of histones in the cytoplasm and have overcome most of these problems. Newly synthesized H4 in the cytoplasm is doubly modified, by an acetate and a phosphate. When cytoplasm is prepared by gentle lysis of cells with a nonionic detergent, then extracted with HCl and the extract freeze dried for electrophoresis on AUT-AUC gels, the pattern of cytoplasmic histone shows doubly modifed H4 as well as the absence of ubiquitin adducts of the H2A's. Thus cytoplasmic histone can be analyzed with little or no contamination from nuclear histone.

We plan to use this newly developed method to study several aspects of the histone pool. The first set of questions concern the relationship of the pool histone to chromatin histone during normal S-phase. Do histones flux in one direction only from polyribosomes, through the pool to chromatin, or do histones flux out of chromatin into the pool? Are histones in the pool degraded? The second set of questions concern how the histones in the pool

react to the inhibition of DNA and/or protein synthesis. Do the levels of histones in the pool change in such a way as to be consistent with autoregulation. The third set of questions concern the level and flux of histone in G1 and GO as well as in S, and the transition between these states.

4. Investigations on Cytoplasmic Histone Pool

Three aspects of the cytoplasmic histone pool in Chinese hamster overy (CHO) cells have been investigated: (A) How are the cytoplasmic levels of individual newly synthesized histones related to the rate of their accumulation in the nuclei at various naturally and artifically regulated stages of cell growth? (B) Do the newly synthesized histones sediment during density gradient centrifugation in the same way as free histone molecules or as complexes of higher molecular weight? (C) How are total cytoplasmic levels of individual histones related to the newly synthesized (labeled) ones?

Cells were labeled for 2 min - 2 hrs with ^{14}C-lysine and radioactive histones in cytoplasm and nuclei were analyzed by fluorography and scintillation counting after two-dimensional gel electrophoretic separation. In the nucleus they accumulate linearily during the first 30 min of incubation and at a slower rate later. The cytoplasmic levels, representing equilibrium between supply and demand, vary greatly depending on the stage and conditions of growth. Low level equilibrium is established in early log phase cells often already after 2 minutes of incubation with only H2A and H2B visible after 1-3 days exposure. In slower growing cells the pool level gets often established at higher values and increases further up to 30 min incubation. H4 is the only one cytoplasmic histone visibly modified. The ratios between double-, single- and nonmodified (acetylated) forms depend on time of incubation and growth stage. After a short (2-5 min) incubation of early log cells the double modified form ($H4b_2$) prevails in cytoplasm. Substantial amounts of both modified forms are found in the nuclei. After longer incubation and in quiescence the equilibrium shifts in the nucleus toward the non acetylated form ($H4b_0$) whereas both modified forms still exist in excess in cytoplasm. In ageing quiescent cells the non modified H4 increases also in cytoplasm at cost of the modified forms.

In cytoplasm centrifuged for 19 hours in a glycerol density gradient (8-24%) labeled histones show up at various levels whereas added non-labeled histone standards are located only in a few top gradient fractions. Broad and overlapping bands of individual histones are detected across the gradient. Besides newly synthesized (labeled) histones, their mass pattern in cytoplasm has been evaluated by Coomassie Blue staining. In the early log cells the only visible spots are of H2A and H2B. In cells growing to confluency spots of all four histones become clearly visible. The pattern of cytopalsmic H4's resembles the nuclear one. Several modifications and improvements in the techniques, mainly in the first dimension electrophoresis, have been introduced.

Significance to Biomedical Research and Program of the Institute:

Cancer at one level is the inappropriate multiplication of cells. Our findings during the last few years have suggested that analysis of histone variant synthesis and the histone variant genes may yield some insight into the relationship of different cell states in normal and neoplastic cells.

Proposed Course:

1. To characterize the histone pool and to test the predictions of the published model as to the behavior of the pool.

2. To develop methodology to study the selective sensitivity of histone mRNA.

Publications:

1. Mueller, R.D., Yasuda, H., Bonner, W.M., and Bradbury, E.M.: Identification of ubiquitinated H2A and H2B in Physarum Polycephalum: Disappearance of these proteins at metaphase and reappearance at anaphase. J. Biol. Chem. 260: 5147-5153, 1985.

2. West, M.H.P., Pantazis, P., and Bonner, W.M.: Studies on nuclease digestion of chromatin phosphorylated in vivo. J. Biol. Chem. 260: 4558-4560, 1985.

3. Sariban, E., Wu, R.S., Erickson, L.C., and Bonner, W.M.: Interrelationship of protein and DNA synthesis during replication in mammalian cells. Mol. Cell Biol. 5: 1279-1286, 1985.

4. Wu, R.S., Panusz, H., Hatch, C.L., and Bonner, W.M.: Histones and their modifications. CRC Crit. Rev. 20: 201-263, 1986.

5. D'Incalci, M., Allamena, P., Wu, R.S., and Bonner, W.M.: H1 variant synthesis in proliferating and quiescent human cells. Eur. J. Biochem. 154: 273-279, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06150-05 LMPH

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Protein-associated DNA Breaks as Indicator of Topoisomerase II Inhibition

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The protein-associated DNA single and double-strand breaks induced in mammalian cells by DNA intercalating agents and epipodophyllotoxins have been shown to represent an effect on topoisomerase II. The aim of this project is to investigate the relationship between the drug-induced protein-associated DNA strand breaks and the physiological state and pharmacological sensitivity of cells. Pharmacological concentrations of epipodophyllotoxins (VP-16 or VM-26) appear to produce only topoisomerase II-mediated DNA breaks in mammalian cells in culture. The association of topoisomerase II with nuclear DNA can be estimated by alkaline elution studies measuring m-AMSA-or VP-16-induced protein-associated DNA breaks. L1210 or DC3F cells seem to have higher levels of topoisomerase II than HT29 or VA-13 cells. Proliferating cells and S-phase cells seem also to have higher topoisomerase II levels than arrested or GO-G1 cells. A good correlation was found between the yields of topoisomerase II-mediated DNA breaks and the cell killing produced by a variety of antitumor drugs. Studies of resistant cells (DC3F/9-OHE) suggest that the sensitivity of cellular topoisomerases II to antitumor drugs could be regulated by other nuclear proteins, such as topoisomerases I.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

1. Determine the mechanism of DNA strand break production by etoposide (VP-16) and teniposide (VM-16) in mammalian cells in culture.

2. Investigate the role of topoisomerase II trapping by DNA intercalators and epipodophyllotoxins in the production of chromosomal aberrations and sister chromatid exchanges in sensitive (DC3F) and resistant (DC3F/9-OHE) Chinese hamster cells.

3. Isolate and compare DNA topoisomerases from sensitive (DC3F) and resistant (DC3F/9-OHE) cells.

4. Determine the cell proliferation and cell cycle-dependence of the m-AMSA- and VP-16-induced protein-associated DNA strand breaks and cytotoxicity.

Methods:

- Standard cell culture and synchronization methods.

- Measurement of DNA strand breaks and protein links by alkaline elution.

- Measurement of sister chromatid exchanges, chromosomal aberrations and cytotoxicity by standard procedures.

- Topoisomerase isolation by gel filtration, DNA affinity columns, glycerol gradient centrifugation (and FPLC).

1. Production of Protein-Associated DNA Strand Breaks by the Epipodophyllotoxin Derivatives Etoposide (VP-16) and Teniposide (VM-26). Relationship to Topoisomerase II Inhibition.

Etoposide (VP-16) and teniposide (VM-26) have been reported to produce protein-associated DNA strand breaks in mammalian cells in culture and to trap topoisomerase II-DNA complexes. VP-16 has also been reported to fragment mammalian DNA without protein-association. This observation suggested that VP-16 could produce free radicals which in turn, could attack DNA. We tested this last possibility by studying the protein-association of the DNA strand breaks produced by VP-16 and VM-26 in three cell lines. HT29 cells are from human colon carcinoma origin and have a low oxidative metabolism. VA13 cells are SV40 transformed human embryonic cells with a high oxidative metabolism and L1210 are mouse leukemia cells in which it was reported that VP-16 produced the two kinds of DNA strand breaks. Both VP-16 and VM-26 produced DNA strand breaks in the three cell lines and in their isolated nuclei. No protein-free breaks were observed but only protein-associated DNA breaks (PASB) whose number was equal to that of DNA-protein crosslinks. VM-26 was more potent than VP-16 at equimolar concentration (5 fold in L1210, 30 fold in VA13, 10 fold in HT29). L1210 cells produced the largest number of PASB at low concentrations of VP-16 or VM-26. VA13 cells produced only slightly more PASB than HT29 cells at low concentrations of VP-16 or VM-26. Taken together, the above results show that 1) neither VP-16 nor VM-26 produce free-radical-mediated DNA strand breaks at concentrations giving less than 2000 rad-equivalents single-strand breaks, and 2) L1210 cells having more drug-sensitive topoisomerase II associated with chromatin than HT29 or VA13 cells.

2. Relationship Between Topoisomerase II Inhibition and Cell Killing by Using 9-aminoacridines Having Different Cytotoxicity

DNA intercalators inhibit topoisomerase II by trapping abortive intermediates of the DNA strand passing reactions of the enzyme. The distribution of these intermediates along the DNA varies from one intercalator to the other, making difficult the comparison of the cytotoxicity of various drugs at equal number of protein-associated DNA strand breaks. This difficulty can be circumvented in the case of the 9-aminoacridine derivatives because they all produce identical DNA cleavage patterns. We then compared the cytotoxicity of various 9-aminoacridines with their potency to produce protein-associated DNA strand breaks in L1210 cells and in isolated nuclei from these cells. A good correlation was found between the two parameters, suggesting that the protein-associated DNA strand breaks, e.g. topoisomerase II inhibition, could be the initial event by which DNA intercalators kill tumor cells.

3. Chromosomal Aberrations Produced by Topoisomerase II Inhibitors in Sensitive and Resistant Cells. Correlation with Cell Killing

We have observed (Pommier et al., Cancer Res., 45: 3143-3149, 1985) that m-AMSA and 5-iminodaunorubicin produce topoisomerase II-mediated DNA strand breaks, chromosomal aberrations and sister chromatide exchanges (SCEs). We also found that the number of SCEs induced by m-AMSA and 5-iminodaunorubicin were correlated with the yield of DNA double-strand breaks produced by the two compounds. The above results were in agreement with the possibility that SCEs could be produced by topoisomerase II-mediated DNA strand exchanges. This hypothesis was further tested by investigating whether the topoisomerase II inhibitors, 2-methyl-9-hydroxyellipticinium (2-Me-9-OH-E+) and etoposide (VP-16) would also produce SCEs and chromosomal aberrations and by comparing the production of chromosomal abnormalities in Chinese hamster cells sensitive (DC3F) and resistant (DC3F/9-OHE) to topoisomerase II inhibitors (Pommier et al., Cancer Res. 46: 611-616, 1986). Three main results emerged from the study: 1) the 3 topoisomerase II inhibitors, VP-16, m-AMSA and 2-Me-9-OH-E+ produced an increased chromosome number, SCEs and chromosomal aberrations in DC3F cells; 2) DC3F/9-OHE cells, that were resistant to the cytotoxic and topoisomerase II effects of the above drugs did not undergo any chromosomal modification upon drug exposure; 3) a good correlation was found between the cytotoxic and the chromosomal effects of topoisomerase II inhibitors in DC3F and DC3F/9-OHE cells. It is therefore tempting to implicate topoisomerase II in the formation of SCEs and to take SCEs as an indicator of cytotoxicity of topoisomerase II inhibitors.

4. Topoisomerase II Modifications in Chinese Hamster Cells Sensitive (DC3F) and Resistant (DC3F/9-OHE) to DNA Intercalators and Etoposide (VP-16)

The resistance of DC3F/9-OHE cells to DNA intercalators and VP-16 involve nuclear modifications that result in a marked reduction of protein-associated DNA strand breaks production (Pommier et al., Cancer Res. 1968). Since there is good evidence that these breaks are topoisomerase II-DNA complexes, we isolated and studied the DNA topoisomerases from sensitive (DC3F) and resistant (DC3F/9-OHE) cells. Topoisomerases purification was performed according to our published procedure (Minford et al., Biochemistry 25: 9-6, 1986). Salt extracts of DC3F/9OHE nuclei did not produce m-AMSA-induced DNA-protein linking but exhibited an unusually high DNA linking activity in the absence of drug.

Fractionation of the salt extract of DC3F/9-OHE nuclei by gel filtration showed two peaks of DNA linking activities. One peak was enhanced by m-AMSA and the other was insensitive to the drug. The m-AMSA-sensitive peak was further fractionated by DNA cellulose chromatography and glycerol gradient centrifugation. It led to the purification of topoisomerases II and I. Both enzymes were similar to those of sensitive (DC3F) cells. They had similar molecular weights, similar DNA topoisomerase activities and similar sensitivity to m-AMSA and VP-16, as judged by their DNA cleavage patterns in sequencing gels. The only difference between DC3F and DC3F/9-OHE topoisomerases II was a two fold lower amount of enzyme, as judged by immunoblotting with rabbit anti L1210 topoisomerase II antibodies. The m-AMSA insensitive peak of DNA-protein linking was also partially purified. It had both DNA linking and topoisomerase I-like activities. The above results suggest that resistant (DC3F/9-OHE) cells have topoisomerase II activities similar to those of sensitive (DC3F) cells but that their topoisomerases II were prevented from being trapped by inhibitory drugs, by the intermediate of DNA binding proteins with topoisomerase I activity.

5. Topoisomerase II Modifications in Relation to Cell Cycle

Mouse 3T3 cells stop proliferation when they reach a certain density on the plate at low serum concentration. Mouse L1210 leukemia cells also stop proliferation at high density. Both in the case of 3T3 and L1210 cells, m-AMSA- and VP-16-induced protein associated DNA breaks were much higher in poliferating than in arrested cells. The fact that the same result was observed in isolated nuclei suggests that high topoisomerase II activity is associated with cell proliferation. Mouse 3T3 cells can be initiated to proliferate by replating at a lower density and feeding with fresh medium containing calf serum. 15 min pulses with [^{3}H]-thymidine showed that such 3T3 cells had a synchronous DNA synthesis wave between 18 and 24 hr. Mitosis occurred between 30 and 35 h. Alkaline elution assays of isolated nuclei treated for 30 min with m-AMSA or VP-16 at various times during this first cell cycle showed that the m-AMSA- and VP-16-induced protein-associated DNA breaks followed the peaks of DNA synthesis. m-AMSA-and VP-16-induced cytotoxicity were also higher in proliferating than in arrested mouse 3T3 cells and the maximuim cytotoxicity was at the DNA synthesis peak during cell cycle. Taken together, these results suggest that topoisomerase II is more closely associated with DNA during DNA synthesis and cell proliferation and that trapping of the enzyme by m-AMSA or VP-16 leads to cell killing.

Proposed Course:

1. Investigate the possibility that purified topoisomerase II produces genetic recombination of plasmid DNA when inhibited by m-AMSA or VP-16.

2. Study the topoisomerase I exhibiting the unusually high DNA linking activity by further purification with FPLC and looking at its effects upon topoisomerase II (topoisomerase II-mediated DNA scission). This could lead to an understanding of topoisomerase II regulatory mechanisms.

3. Investigate the mechanism(s) by which the reversible protein-associated DNA strand breaks (topoisomerase II inhibition) produced by intercalators and epipodophyllotoxins lead to cell death.

Publications:

1. Pommier, Y., Zwelling, L.A., Kao-Shan, C.S., Whang-Peng, J., and Bradley, M.O.: Correlations between intercalator-induced DNA strand breaks, sister chromatide exchanges, mutations and cytotoxicity in Chinese hamster cells. Cancer Res. 45: 3143-3149, 1985.

2. Pommier, Y., Schwartz, R.E., Zwelling, L.A., and Kohn, K. W.: Reduced formation of protein-associated DNA strand breaks in Chinese hamster cells sensitive and resistant to topoisomerase II inhibitors. Cancer Res. 46: 611-616, 1986.

3. Pommier, Y., Kerrigan, D., Schwartz, R.E., Swack, J., and McCurdy, A.: Altered DNA topoisomerase II activity in Chinese hamster cells resistant to topoisomerase II inhibitors. Cancer Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06161-03 LMPH

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 cherecters or less. Title must fit on one line between the borders.)*

Topoisomerase II as Target of Action for Anticancer Drugs

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principel Investigetor.) (Name, title, leboratory, and institute affiliation)*

PI:	Yves Pommier	Visiting Associate	LMPH NCI
Others:	Donna Kerrigan	Chemist	LMPH NCI
	Judith Markovits	Guest Researcher	LMPH NCI
	Joseph Covey	Staff Fellow	LMPH NCI
	Kurt W. Kohn	Lab Chief	LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the spece provided.)*

DNA topoisomerase II and I can be purified to homogeneity from mouse L1210 and Chinese hamster (DC3F; DC3F/9-OHE) cells. The purification procedure is based upon the detection of topoisomerase II by its DNA linking and breaking activity in the presence of m-AMSA. Purified topoisomerase II produced protein-associated DNA breaks in the presence of the same compounds that produce protein-associated DNA breaks in cells. In addition, in both cases the breaks do not allow DNA swivelling, their 5'-termini are covalently linked to the enzyme and their formation can be inhibited by high concentrations of 2-methyl-9-hydroxyellipticinium. It is therefore likely that topoisomerase II is the intracellular target of m-AMSA, anthracyclines, ellipticines and epipodophyllotoxins, by the intermediate of which they produce the protein-associated DNA breaks. Cyanomorpholinoadriamycin has a unique mode of interaction with DNA involving DNA interstrand crosslinks and no (or weak) antitopoisomerase II activity. m-AMSA seems to interact directly with topoisomerase II by the intermediate of its side chain. DNA intercalation is not sufficient to affect topoisomerase II. For example, 9-aminoacridine is a better intercalator than m-AMSA but has no (or weak) antitopoisomerase II activity. Polyamines affect topoisomerases activities and could play a role in controlling DNA topoisomerases in mammalian cells.

PHS 6040 (Rev. 1/84) GPO 914-918

Objectives:

1. Improve the purification procedure of topoisomerase II.

2. Study the modes of interaction of the adriamycin analogs, cyanomorpholino-adriamycin and morpholino adriamycin with DNA and topoisomerase II.

3. Study the mechanism by which m-AMSA and other 9-aminoacridines trap topoisomerase II-DNA complexes and determine the nucleotides sequences around the DNA cutting sites.

4. Determine the effects of polyamines (spermine & spermidine) and non-intercalative DNA binding drugs (distamycin A and anologs) upon topoisomerase II activities.

Methods:

1. Purification of topoisomerases from mammalian cell nuclei by gel filtration, DNA cellulose chromatography and glycerol gradient centrifugation. Application of FPLC to topoisomerase purification.

2. Assays of topoisomerase activities: filter binding assays to detect DNA-protein links, gel electrophoresis of catenated supercoiled or [^{32}P]-end labeled linear DNA to detect DNA topoisomers and fragments.

Major Findings:

Purification of DNA Topoisomerases from Mammalian Cells in Culture

Three assays can be used to detect DNA topoisomerase II without interference of nucleases or topoisomerase I: 1) Filter binding assay looking for m-AMSA-dependent DNA linking activity (Minford et al., Biochemistry 25: 9-16, 1986), 2) cleavage assay by agarose gel electrophoresis of supercoiled SV40 DNA looking for m-AMSA-induced DNA cleavage and, 3) decatenation assay of crithidia fasciculata DNA looking for ATP-dependent DNA decatenation.

Using these three assays for enzyme activity we can purify topoisomerases II and I from mouse L1210 and Chinese hamster lung DC3F and DC3F/9-OHE cells. The purification steps are: 1) making nuclei by Triton X-100 solubilization of plasma membranes, 2) extraction of soluble nuclear proteins in 0.35 M NaCl, 3) fractionation of the nuclear extract by Sephacryl S-400 gel filtration, 4) further purifiction of the active fractions by denatured DNA cellulose chromatography and 5) glycerol gradient centrifugation.

Purified mammalian topoisomerase II appear as a cluster of protein bands around 170 KDa in SDS/PAGE gel and has a S value of 11 in glycerol gradients. Topoisomerase I has a M.W. around 100 KDa in SDS/PAGE and a S value of 6.5. Two observations can be made about our purification procedure: 1) topoisomerases II and I copurify up to the glycerol gradient step, suggesting that the two enzymes could be part of the same complexes; 2) the enzymes that we purify are only those that are not released from nuclei at physological salt concentration but are soluble in 0.35 M NaCl. Therefore we probably extract only a subset of the cellular topoisomerases.

DNA Effects of Cyanomorpholinoadriamycin in the Absence or Presence of L1210 Topoisomerase II. Comparison with Morpholino-Adriamycin and Adriamycin

Cyanomorpholinoadriamycin (CNM_0D) is extremely cytotoxic (at nanomolar concentrations) towards mammalian cells, whereas morpholinoadriamycin (M_0D) and adriamycin (ADR) are cytotoxic at much higher concentrations. The reasons for this difference were investigated by comparing the effects of the compounds upon purified DNA and topoisomerase II activity. Incubation of [^{32}P]-end labeled pBR 322 DNA with CNM_0D retarded the DNA migration. This effect was observed at micromolar concentrations in non-denaturing and DNA denaturing conditions (neutral and alkaline agarose gel electrophoresis) and in the presence of SDS, which suggests that CNM_0D produces DNA interstrand crosslinks. Neither M_0D nor ADR produced DNA interstrand crosslinks in these conditions. The effects of CNM_0D and M_0D upon L1210 topoisomerase II-induced DNA cleavage were also investigated. Neither of the compounds enhanced topoisomerase II-induced DNA cleavage. The above results suggest that CNM_0D has a unique mode of interaction with DNA involving the formation of DNA interstrand crosslinks and no or very weak effects upon mammalian topoisomerase II. The DNA crosslinking effect of CNM_0D could therefore be responsible for its unusually high cytotoxicity.

Role of Intercalation in Topoisomerase II Inhibition by m-AMSA and Other 9-aminoacridines

m-AMSA (4'(9-acridinylamino)methanesulfon-m-anisidide) is one of the most potent topoisomerase II inhibitors both in mammalian cells in culture and in purified systems. The interaction of m-AMSA with topoisomerase II-DNA complexes is, however unknown. In order to study this question, we have used various analogs that we obtained from B. Baguley (New-Zealand Cancer Research Laboratory). The DNA intercalative effects of the aminoacridine derivatives was first studied by a topoisomerase I unwinding assay (Pommier et al., Biochemistry 24: 6410-6416, 1986). The derivatives studied varied markedly: for example, 9-aminoacridine was 6 fold, and o-AMSA 2 fold more potent than m-AMSA with respect to intercalation. The potency of the 9-aminoacridine derivatives to inhibit topoisomerase II was, however very different: at equal intercalative concentrations, m-AMSA was more potent than o-AMSA, which, in turn was more potent than 9-aminoacridine. It is therefore clear that intercalation of acridine derivatives is not a sufficient requirement for topoisomerase II trapping. More likely the presence of a bulky side group in position 9 of the acridine ring seems necessary and the stereochemistry of this bulky group is important since m-AMSA and o-AMSA have such different effects. Another argument against the critical role of DNA intercalation comes from the observation that the DNA cleavage patterns and the sequence localization of the DNA cuts of 9-anilinoacridine derivatives having either A-T or G-C preferential DNA bindings, are similar. Taken together, our results suggest a direct interaction between active 9-aminoacridines and topoisomerase II.

Effects of Polyamines Upon L1210 Topoisomerase II

Polyamines (putrescine, spermidine, spermine) are found at millimolar concentrations in mammalian cells. They are known to bind to the minor groove of duplex DNA and to affect DNA functions. In order to investigate this last

aspect, we decided to study the effects of spermine(S) upon topoisomerase II. Two opposite effects were found depending on the concentration. At 10^{-5}-10^{-4}M, S stimulated the formation of topoisomerase II-DNA complexes and the DNA relaxing activity of the enzyme without increasing the topoisomerase II-mediated DNA cleavage. At 10^{-3} M and above, both the formation of topoisomerase II-DNA complexes and the topoisomerase II DNA strand passing activity were inhibited. By analyzing the effects of S upon the localization of topoisomerease II-induced DNA breaks in [^{32}P]-end labeled DNA, it appeared that S had no effect at concentrations below 10^{-3}M and an inhibitory effect at 10^{-3}M. However some cleavage sites were specifically enhanced at this concentration both in the absence or presence of m-AMSA. Spermidine had only the stimulatory effect up to 10^{-3}M. The above results suggest a regulatory role of polyamines upon topoisomerase II activity.

Proposed Course:

1. Improve the actual topoisomerase II purification procedures by using FPLC. Increasing the yield should allow easier purification with less cellular material. This would be useful in comparing various states of the enzyme under various cellular conditions and to raise antibodies against L1210 topoisomerase II.

2. Look at topoisomerase II binding sites on purified DNA by footprinting methods. Compare the enzyme binding sites with its DNA cleavage sites.

3. Study the effects of minor groove binders, such as distamycin A and netropsin upon topoisomerase II-induced DNA reactions (topoisomerization, DNA binding, DNA cleavage in the absence or presence of intercalators).

Publications:

1. Pommmier, Y., Schwartz, R.E., Zwelling, L.A., and Kohn, K.W.: Effects of DNA intercalating agents on topoisomerase II-induced DNA strand cleavage in isolated mammalian cell nuclei. Biochemistry 24: 6406-6410, 1985.

2. Pommier, Y., Minford, J.K., Schwartz, R.E., Zwelling, L.A., and Kohn, K. W.: Effects of the DNA intercalators, 4'(9-acridinylamino)methanesulfon-m-anisidide and 2-methyl-9-hydroxyellipticinium or topoisomerase II-mediated DNA strand cleavage and strand passage. Biochemistry 24: 6410-6416, 1985.

3. Minford, J.K., Pommier, Y., Filipski, Y., Kohn, K.W., Kerrigan, D., Mattern, M.R., Michael, S., Schwartz, R.E., and Zwelling L.A.:. Isolation of intercalator-dependent protein-linked DNA strand cleavage activity from cell nuclei and identification as DA topoisomerase II. Biochemistry 25: 9-16, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06170-02 LMPH

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Isolation of Human Variant Histone Genes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Bonner	Head, Chromosome Structure and Function Section	LMPH NCI
Others:	Christopher Hatch	Staff Fellow	LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION
Chromosome Structure and Function

INSTITUTE AND LOCATION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objective is to isolate the genes for the human histone H2A family, in particular the H2A.Z gene.

A number of putative H2A.Z clones have been isolated and are being further screened.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description

Introduction:

The regulation of the various histone variants differs greatly with respect to the rates of protein and DNA synthesis. Isolation of the genes for the variant histones will help elucidate these regulatory mechanisms.

Objective:

To isolate human histone genes particularly the gene for H2A.Z.

Methods:

1. Recombinant DNA techniques.

2. Use of synthetic probes to prime reverse transcriptase and search genomic libraries.

3. Rapid RNA methodology developed in this laboratory.

Major Findings and Accomplishments:

In order to isolate the various histone genes, we have utilized the human genomic library originally established in Phil Leder's laboratory by the insertion of human placental DNA into the charon 28 lambda bacteriophage. A "complete" library of the human genome in lambda was grown with C600 E. coli bacteria and plated in 15 cm diameter plates. Each plate was replica plated in duplicate with sterile nitrocellulose filters. The filters were processed and incubated with radioactively labeled DNA probes.

Our primary effort has been towards the isolation of the genes encoding the minor variant form of H2A, H2A.Z. The isolation of non-S-phase histone genes has been difficult. This problem is most acute in the case of the gene for histone H2A.Z., since the amino acid sequence of this polypeptide has been found to be quite divergent from that of the other histone H2A sequences while it appears that the amino acid sequence of H2A.Z is conserved in a wide variety of species, the nucleotide sequences encoding this polypeptide may be more divergent between species. An H2A.Z related gene, called H2A.F, has been isolated from chicken, but cross-hybridization of this gene to homologous sequences in human, mouse, or sea urchin DNA was not found. This demonstrated that rather divergent nucleotide sequences could be coding for the same protein sequence and reinforced our belief that it would be difficult to utilize heterologous histone H2A gene probes to search for the human H2A.Z gene sequence. A more general approach not subject to these drawbacks involves the preparation of probes from known amino acid sequences. These specific probes for the detection of the desired histone mRNA's or genes can be obtained by the chemical synthesis of oligonucleotides which represent all possible codon combinations for a short run of amino acids in a given protein. H2A residues 24-28 were of particular interest to us since it has been determined that all H2A histone variants which had been sequenced in plants and animals have a common amino acid sequence from residues 21 to 29. We designed a 14-nucleotide length oligonucleotide sequence mixture that would contain

sequences in this region. The probe designed contained a mixture of 14-base long synthetic oligonucleotides containg 16 possible combinations of sequence.

In order to both increase the specificity and stability of hybridization between the designed probe and the homologous sequences within the human genomic lambda library the synthetic oligonucleotide mixture was utilized as a primer for making partial cDNA copies of mRNA onto which it hybridized. In order to increase the proportion of H2A.Z mRNA relative to the other H2A mRNA's the mRNA treated with hydroxyurea for one hour prior to RNA purification from these cells. This treatment reduces the amount of major H2A variant mRNA and therefore would effectively increase the relative proportion of minor H2A variant mRNA. The oligonucleotide probe mixture was used to prime synthesis of radioactive cDNA copies of template mRNA. The ^{32}P-labeled cDNA's were then used to probe the human genomic lambda library. On the order of 1/500-1/1000 of the human-lambda clones were observed to hybridize the radioactive probes.

We decided to make a new synthetic probe mixture complementary to the mRNA encoding amino acid residues #4 through #8 of H2A.Z and H2A.F. A 14-base long synthetic probe mixture with 32 different possible sequence combinations was designed and commercially synthesized. Both this probe and the previously utilized 14-mer probe mixture were used to make ^{32}P-labeled cDNA probes. These probes were found to specifically hybridize to the correct H2A.F gene-containing DNA fragments when the restriction enzyme digestion and hybridization analysis was done. Each of these probes were used to probe duplicate nitrocellulose replicas of the human genomic-lambda library. The autoradiographs of the filters from each plate were aligned to see which plaques showed positive hybridization to both of these independent probes. Although many plaques were doubly-positive, a smaller fraction appeared to match quite well in signal intensity. The plaques corresponding to the best matches were isolated, the phage was grown, the DNA was isolated, digested with restriction enzymes, run on agarose gels, and southern-transfered onto nitrocellulose. A number of human-lambda clones were identified as containing similar patterns of restriction enzyme-produced DNA fragments that were labeled by hybridization to the above two ^{32}P-labeled primer-extension cDNA probes. The chicken H2A.F gene plasmid (obtained from Prof. Julian Wells of Adelaide, Australia) was then uitilized in a effort to narrow the number of positive clones. However, it was found that a ^{32}P-labeled-restriction fragment containing only the H2A.F gene cross-hybridized with most of the lambda clones selected by the primer-extension-cDNA probes. So although this gene provided some confirmation of the selection by the cDNA probes it provided no further selection. We therefore had a new synthetic probe constructed which takes advantage of recently developed technology for the insertion of deoxyirosine into codon wobble positions. This allowed the original synthetic probe designed to be complementary to the gene sequence encoding H2A.Z (and H2A.F) amino acids #4-8 to be extended all the way to the amino terminal end of the protein coding sequence, thusly allowing the oligonucleotide probe for this region to be increased in length from 14 to 23 nucleotides in length. This probe is presently being used for direct probing of the pre-selected clones. By using the probe in this way we hope to eliminate the probe heterogeniety that is inevitable when an oligonucleotide is used to make a cDNA probe.

Attempts to use hybrid selection and in vitro translation to distinguish the true positive clones from those that gave false positive hybridization signals have so far proved unsuccessful due to the inherent poor translatability of the H2A.Z mRNA in the available in vitro translation systems. We did not know whether this might also be due to very low amounts of this messenger RNA normally being present in cells or to its instability during the preparation of total cellular RNA for hybrid selection in vitro translation assay. We began to investigate these questions and in so doing have developed a comprehensive methodology for the rapid analysis of the levels of particular mRNA's in whole cell and cytoplasmic extract preparations. We have utilized this methodology to analyze the H2A.F mRNA in chicken cells and are similarly analyzing the homologous H2A.Z mRNA of mammalian cells. This affords us several new approaches. First, clones which contain DNA that hybridizes selectively to the putative H2A.Z mRNA band can be selected for further analysis. Second, we hope to be able to construct cDNA clone populations which are highly enriched in copies of the H2A.Z mRNA after isolation of the putative messenger RNA band from methylmercury gels.

It therefore appears that human-lambda DNA clones have been found which hybridize selectively to two independent probes for the H2A.Z gene.

This project is now at the crucial stage of determining whether or not the DNA of the chosen human-lambda clones can be used successfully to hybrid select and in vitro translate the mRNA encoding H2.Z.

Significance:

Isolation of histone genes of the H2A family would permit studies into the molecular basis of the different regulation of histone variants relative to DNA replication.

Proposed Course:

1. To test the doubly positive clones with an H2A.F gene (probably related to H2A.Z) from chicken (donated by Prof. Julian Wells.)

2. To construct a synthetic probe using recently developed technology of inserting inosine into wobble positions. A longer probe could be constructed which could be used to search a genomic library directly.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06171-02 LMPH

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Chromatin Synthesis and the Control of Cell Proliferation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Bonner	Head, Chromosome Structure and Function Section	LMPH NCI
Others:	Roy S. Wu	Expert	LMPH NCI
	Christopher Hatch	Staff Fellow	LMPH NCI

COOPERATING UNITS *(if any)*

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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Our objective is to understand the relationship of various cell states and the transitions between them as reflected in the control of histone and chromatin biosynthesis. One phenomenon which we have studied is the differential sensitivity of the growth and chromosome cycles to the rate of protein synthesis. The chromosome cycle seems to be relatively invariant even though other results show that DNA synthesis is inhibited in parallel to the inhibition of protein synthesis. To resolve this apparent paradox, we studied histone and DNA synthesis under different conditions of protein synthesis and cell growth.

We have found and characterized a phenomenon which we have named chromosome cycle compensation. As protein synthesis is inhibited, the mRNA level of S-phase histones rises to compensate for the inefficiency of protein synthesis. There is evidence in the literature that normal and tumorigenic cells may differ significantly in their level of chromosome cycle compensation. Understanding this mechanism may lead to some insights into the growth characteristics of normal and tumorigenic cells.

Project Description

Introduction:

The biochemical mechanisms which regulate cell growth remain largely unknown. For example it has been widely documented that as cell growth is slowed, the chromosome related activities and cell cycle phases are relatively unaffected. The biochemical basis for the phenomenon was unknown. Our studies this year have resulted in an explanation for this phenomenon in CHO cells.

Objective:

1) The development of a greater understanding of the relationship of the mechanisms regulating chromatin biosynthesis to the molecular mechanisms regulating cell proliferation.

2) A characterization of the responses of these regulatory mechanisms to the development of new regimens or compounds that might control inappropriate proliferation of transformed cells.

Methods:

(1) Discontinuous electrophoretic separation of histones including direct loading of histone extracts and two dimensional electrophoresis. (Methods developed in this laboratory).

(2) Synchronization of cell lines, particularly human Hela cells and Chinese hamster ovary cells for studies on cell cycle.

(3) Maintenance of cells and nuclei in viable non-dividing states using modified and defined media.

(4) Isolation and analysis of mRNA from different parts of the cell cycle or from quiescent cells, using methodology developed in concurrent project.

(5) FACS analysis of cell cycle distributions including the BrdU antibody technique for measuring S phase cells.

Major Findings and Accomplishments:

Relationship Between Protein Synthesis Inhibition, the Chromosome and Growth Cycles and the Levels of Histone mRNA and Chromatin Synthesis: An Explanation for the Relative Invariance of the Chromosome Cycle

The information reported in the literature concerning the regulatory mechanisms between DNA and protein synthesis has generally been obtained using high concentrations of inhibitors which slow either DNA or protein synthesis to 1-5% of their control level and which prevent cell growth. During the course of our work with protein synthesis inhibitors we noticed that our results on the regulation of chromatin biosynthesis around the cell cycle presented us with an intriging paradox. That is, the rate of cell growth (G_1 + S + G_2 + M) was apparently inhibited in proportion to the inhibition of protein synthesis but the rate of progression of cells through the chromosome cycle (S + G_2 + M)

apparently was not. To attempt to resolve this paradox, we examined the effect of the rate of protein synthesis on the rates of synthesis of the two major components of chromatin, histone proteins and DNA, in CHO cells. We found that immediately after the inhibition of protein synthesis with low doses of cycloheximide, total protein, histone protein and DNA syntheses were inhibited proportionately. However, within a few hours the rates of synthesis of S-phase histones and DNA increased relative to the rates of synthesis of non-histone proteins and certain basal variants. Thus, proliferating CHO cells in S-phase have the ability to sense changes in the rate of protein synthesis and can compensate for any decreases in the rates of chromatin replication by increasing specifically the rates of synthesis of the two major components of chromatin, histone proteins and DNA. We call this process chromosome cycle compensation. The mechanism for this compensation involves a specific increase in the level of histone mRNAs in response to the decreased rate of protein synthesis. The amount of compensation seen in CHO cells can account quantitatively for the relative invariance in the length of the chromosome cycle ($S + G_2 + M$) reported for these cells. An invariant chromosome cycle sampled with a lengthening growth cycle ($G_1 + S + G_2 + M$) must result in a disproportionate lengthening of the G_1, phase. Thus, these results suggest that chromosome cycle invariance may be due more to specific cellular compensation mechanisms rather than the more usual interpretation involving a rate limiting step for cell cycle progression in the G_1 phase ordinary known as G_1, cell cycle arrest or G_1 restriction point.

Cycloheximide shows the elongation step of translation and as such may not be a natural method for regulating protein synthesis. We are currently studying more physiological methods of limiting growth such as amino acid starvation and serum deprivation to reduce growth factors. Preliminary evidence suggest that histone mRNA levels can vary in response to changes in amino acid as well as growth factor levels in the medium.

There is also some evidence in the literature which suggests that there may be significant differences in the regulation of chromatin biosynthesis between normal and tumorgenic cells. Currently we are comparing the effects of protein synthesis inhibition on a family of 3T3 cell lines, A31, SV40 transformed A31, benzpyrene transformed and MNNG transformed 3T3 cells, to see whether any differences in the chromosome cycle compensating mechanism can be observed. Preliminary evidence suggest that there are big differences in the ability of A31 and SV40 transformed A31 cells to compensate for protein synthesis inhibition. Understanding these differences may lead to the development of protocols to protect normal cells or make tumorgenic cells more vulnerable to various treatments.

Significance:

There are suggestions in the literature that normal and tumorgenic cells respond very differently in terms of cell cycle distribution when their growth is slowed. Our findings provide a molecular basis for this effect and thus provides experimental framework for studying this phenomenon.

Proposed Course:

1. To characterize the effect of slow growth on chromosome cycle duration in several cell lines, particularly matched pairs of normal and transformed lines, such as mouse 3T3 and BP-3T3 or human IMR-90 and VA-13.

2. To test whether chromosome cycle compensation exists in these cells or whether there are significant differences.

Publications:

1. Wu, R.S. and Bonner, W.M.: Mechanism of differential sensitivity of the chromosome and growth cycle of mammalian cells to the rate of protein synthesis. Mol. Cel. Biol. 5: 2959-2966, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06172-02 LMPH

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October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Base Sequence Selective DNA Alkylation Reactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	Kurt W. Kohn	Lab Chief	LMPH NCI
Others:	William B. Mattes	Staff Fellow	LMPH NCI
	John Hartley	Visiting Fellow	LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

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SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
3.0	2.1	0.4

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are continuing a project investigating the DNA base-sequence selectivity of alkylation reactions. The main objective is to determine whether base-sequence selectivities contribute to the anti-tumor activities of alkylating agents and whether the effectiveness of these drugs could be enhanced by structural modifications that would optimize the selectivity for certain sequences. DNA sequencing methodology was used to localize alkylations at guanine-N7 positions in a variety of procaryotic and eucaryotic DNA sequences. Marked differences in reaction intensities were observed at different guanines in a DNA sequence, as well as between different nitrogen mustards. Some of the differences in reaction intensities at different guanines may be due to sequence-dependent variations in the electrostatic potential at the guanine-N7 position. However, some nitrogen mustards, notably quinacrine mustard and uracil mustard have, in addition, unique and specific reactions at certain DNA sites. These and other sequence-dependent patterns are being investigated quantitatively.

PHS 6040 (Rev. 1/84)

GPO 914-918

Project Description

Objectives:

1. Determine the base sequence selectivities for the reactions of alkylating agents with DNA.

2. Investigate the structural and conformational dependences of the selectivities.

3. Devise new alkylating agent structures with enhanced base sequence selectivities, especially those targeted for certain oncogenes.

Methods:

1. High-resolution polyacrylamide gel electrophoresis of end-labeled DNA restriction fragments previously reacted with alkylating agents and chemically cleaved at sites of guanine-N7 alkylation.

2. Quantitative densitometry and computer analysis of electrophoretic gel autoradiograms.

Major Findings:

Reaction of Nitrogen Mustards at Guanine-N7 Positions

One of our early observations when we began this project in early 1984 was that mechlorethamine and melphalan had a relatively strong preference for alkylating guanines that are flanked in a DNA sequence by other guanines, i.e. guanine runs. This observation prompted a search of the GENBANK DNA sequence data base for DNA sequences containing large runs of Gs or Cs. A search for sequences 30 bases long containing at least 28 Gs or Cs turned up 50 genetic loci, 20 (40%) of which qualify as oncogenes or transforming virus. Of note is the fact that genes classified as oncogenes constitute only 5% of the primate, vertebrate and viral sequences; perhaps GC rich regions serve a special role in oncogene expression or regulation. Additionally, our data indicate that such regions may be preferred sites of alkylation by nitrogen mustards and nitrosoureas and perhaps important chemotherapeutic targets.

We have extended our findings from the previous year, i.e. that there are marked differences in reactivity among guanines in a DNA sequence and that the nature of the non-alkylating moiety of a nitrogen mustard plays a role in determining the sequence selectivity of alkylation. One feature common to almost all of the nitrogen mustards is a preferred alkylation at runs of guanines. This may arise from an electrostatic interaction between the positively charged aziridinium of the nitrogen mustard and an increased electronegativity at the guanine-N7 position in such sequences. Indeed, there seems to be a good correlation between the sequence-dependent variations in alkylation intensity by simple nitrogen mustards and the sequence-dependent variations in electrostatic potential at the guanine-N7 position. In keeping with this notion, we find that the preference of simple nitrogen mustards for reaction at runs of guanines is reduced by an increase in ionic strength of the

reaction buffer. We also find that chloroethylating agents have a marked preference for reaction at runs of guanines; we have suggested that a partial chloronium ion with a positive charge may interact electrostatically with such sequences in a fashion similar to that of the nitrogen mustards.

On the other hand, the sequence selectivities of quinacrine mustard and uracil mustard are in contrast with those of mechlorethamine and melphalan. The alkylation of DNA in vitro by quinacrine mustard shows a preference for runs of guanines almost to the exclusion of isolated guanines, a preference that is not appreciably affected by ionic strength. Quinacrine mustard also shows some unique and predictable sequence preferences for certain isolated guanines, which we have determined by use of computer aided densitometric analysis. Thus we think that the acridine portion of this compound is directing its alkylation. Uracil mustard shows a remarkable preference for alkylating 5'-TGCC-3' sites: this preference is eliminated by a 6-methyl or N-1 methyl group. We are presently investigating the uracil mustard- DNA interactions with model building.

Significance:

1. These studies may lead to the development of new alkylating agents with high base-sequence selectivities, especially for certain oncogene sequences.

2. The studies may also yield new information about the structure and conformations of DNA in solution.

Proposed Course:

1. Carry out quantitative densitometry and computer analyses of the sequence-dependence of the reactions of a wide variety of nitrogen mustards.

2. Carry out molecular graphics analyses in order to obtain structural hypotheses to account for the observed selectivities. Test these hypotheses by synthesis of new nitrogen mustard structures that would be predicted to show strong selectivities.

3. Extend these studies to DNA in chromatin and intact cells..

Publications:

1. Hartley, J.A., Gibson, N.W., Kohn, K.W., and Mattes, W.B.: DNA sequence selectivity of guanine-N7 alkylation by three antitumor chloroethylating agents. Cancer Res. 46: 1943-1947, 1986.

2. Mattes, W.B., Hartley, J.A., and Kohn, K.W.: DNA sequence selectivity of guanine-N7 alkylation by nitrogen mustards. Nucleic Acids Res. 14: 2971-2987, 1986.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06183-01 LMPH

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effects of Incorporation of Base Analogs into DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Joseph Covey Staff Fellow LMPH NCI

Others: Eugene J. Tilchen IPA LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Molecular Pharmacology

SECTION

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

While the effects of cytotoxic antimetabolites on DNA precursor synthesis and DNA replication have been extensively studied and in some cases related to the anti-tumor activities of these agents, the effects of antimetabolites on DNA structure and integrity have not been as well characterized. The focus of this project is to investigate the nature and extent of DNA damage produced by several antimetabolites (5-aza-2'-deoxycytidine, aza-dC; 5,6-dihydro-5-azacytidine, H_2-aza-C; 6-thio-guanine, TG) using the alkaline elution technique, and to determine the relation-ship of this damage with cytotoxicity. We have shown that aza-dC produces alkali-labile sites in L1210 DNA which has incorporated the drug. These lesions are removed slowly with no decrease in the first 24 h after drug washout. The DNA damage may arise through the chemical instability of the azacytosine ring, which, when incorporated into DNA, leads to strand scission in alkaline solution. In contrast, the stable analog H_2-aza-C produces DNA single-strand breaks (SSB) with no alkali dependence. H_2-aza-C was also shown to have a potent inhibitory effect on DNA elongation and/or ligation, while aza-dc does not. Substantial repair of H_2-aza-C SSB occurs after 24 h. These observations suggest that aza-dC and H_2-aaz-C have markedly different effects on DNA integrity and that the two agents should not be considered simple pharmacologic congeners. TG has been shown to produce a moderate level of SSB in cultured cells, an observation confirmed in our studies. We have shown, however, that TG induces DNA-protein crosslinks (DPC) at a frequency far in excess of SSB. DPC were produced in both the daughter DNA strand (i.e., the strand which has incorporated the drug) and in the parental strand. The lesions could be prevented by co-incubation with hydroxyurea and reversed by treatment with proteinase-K. It is proposed that TG in DNA becomes linked to protein(s), perhaps via a disulfide bond. If this occurs at or near the point of DNA replication, trapping of the template in a frozen replication complex may explain the parental lesions. These observations are consistent with the delayed cytotoxicity and unilateral chromatid damage which has been reported for TG.

Introduction

A number of base or nucleoside analogs with demonstrated antitumor activity are known to inhibit DNA synthesis. This activity generally requires anabolsm of the analog to the deoxyribo- (or arabino-) nucleotide or other metabolic intermediates. These activated species can either inhibit DNA synthesis directly or have an indirect effect through perturbations in the synthesis of DNA precursors. Many of these antimetabolites are also incorporated into DNA, a process which can lead to macromolecular DNA damage. Thus the biochemical effects of these analogs are likely to be multiple and complex. While effects on DNA synthesis have been extensively studied and in some cases related to cytotoxicity, the consequences of DNA damage are less well characterized. It is the focus of these studies to investigate the effects of several antimetabolites on DNA integrity using the alkaline elution technique.

Objectives:

1. To determine the nature and extent of DNA damage produced by antimetabolites which are known to be incorporated into DNA.

2. To elucidate the mechanisms by which these analogs produce the observed DNA lesions.

3. To relate the effects on DNA integrity to the known biological, morphological and cytotoxic properties of these compounds.

Methods:

1. Standard cell culture techniques; viability determination by soft-agar cloning

2. Measurement of DNA single-strand breaks (SSB) and DNA-protein crosslinks (DPC) by alkaline elution.

3. Measurement of drug effects on DNA elongation using pulse radiolabeling and sequential alkaline elution.

Major Findings and Accomplishments:

1. Comparison of DNA Damage Produced by Incorporation of 5-Aza-2'-deoxycytidine (aza-dC) or 5,6-Dihydro-5-azacytidine (H_2aza-C) into DNA of Mammalian Cells

Aza-dC, a compound with demonstrasted clinical activity in childhood leukemia, has been shown to be incorporated into DNA and to produce a potent inhibition of DNA-cytosine-methyltransferase. This effect results in hypomethylation of DNA and can lead to changes in cellular gene expression. While the inhibition of methylation has been correlated with aza-dC cytotoxicity in L1210 cells, these effects can be disassociated in other systems, and it remains unclear if this is the unique mechanism of antitumor activity for this agent.

Using the alkaline elution technique we have demonstrated that treatment of L1210 cells with aza-dC (0.1-10 µg/ml) results in a concentration dependent production of alkali-labile sites (DNA lesions which are converted to SSB in alkali). These lesions are confined to the DNA segments which have incorporated the drug

(simultaneous labeling), since no damage was detected in pre-labeled cells. The DNA damage persists following drug removal, with no decrease observed at 24 h and only a moderate decline at 72 h. Since aza-dC is unstable in aqueous solution, undergoing a ring-opening reaction, (the rate of which is accelerated by alkali), we proposed that DNA strand-scission results from the presence of ring-opened moieties in DNA, which leads, at high pH, to cleavage of the 3'-sugar-phosphate bond through a reaction sequence analogous to the behavior of base-free sites under alkaline conditions. Since the rate of aza-dC ring opening may be reduced when the base is incorporated into DNA, it remains unclear if the lesions present in native DNA in vivo represent simply sites of misincorporated base or its degradation products.

In an attempt to further elucidate the mechanism of aza-dC induced DNA damage, studies were also done using the chemically stable analog H_2-aza-C. This compound is significantly less potent against L1210 in vitro and in vivo, but has undergone Phase I clinical trial. As measured by alkaline elution, H_2-aza-C produced a significant and dose-dependent (1-100 µg/ml) frequency of DNA SSB in L1210 cells, with no evidence of alkali-labile behavior. These lesions were observed to undergo significant repair after 24 h of drug-free incubation. The contrasting pattern of DNA damage and repair produced by aza-dC and H_2-aza-C in L1210 was also observed in two human lymphoblastoid cell lines, one of which was derived from a patient with xeroderma pigmentosum (XP). The lack of significant differences in the rate of repair of H_2-aza-C-induced lesions in these two cell lines suggests that the repair defect present in the XP line does not play a role in the removal of H_2-aza-C lesions.

To investigate the possibility that aza-dC or H_2-aza-C-induced DNA damage resulted from an inhibitory effect on DNA elongation secondary to drug incorporation, the growth of pulse-labeled DNA segments was examined using sequential alkaline elutions. Control cells and cells treated with aza-dC for 1h (1-25 µg/ml) following a 15 min [^{3}H]-thymidine pulse grew to full length over the next 7 h. In contrast, cells treated by the same protocol with ara-C (12 µg/ml), a compound with known chain terminating properties, demonstrated a marked slowing of elongation. Somewhat unexpectedly, treatment with H_2-aza-C (50 µg/ml) resulted in a complete cessation of DNA elongation for at least 4 h following drug removal.

These studies have demonstrated contrasting DNA damaging effects for aza-dC and H_2-aza-C. Since the latter compound was synthesized as a stable analog for clinical development, it is important to recognise that the two agents cannot be considered a simple pharmacologic congeners, but rather must be treated as unique entities with potentially different mechanisms of cell killing.

2. Production of DNA-Protein Crosslinks by 6-Thioguanine in L1210 Cells

The purine analog TG has recently been shown to produce DNA SSB, unilateral chromatid damage and delayed cytotoxicity in several cell lines (L1210, CHO) following in vitro exposure. We have investigated the formation of DNA-protein crosslinks in L1210 cells treated with TG or TGdR, using the alkaline elution technique. When cells were either prelabeled with [^{14}C]thymidine (18-24 h), washed and chased (1-24 h) and then treated with TG (0.25-4 µM) for 1-24 h (SEQ protocol) or labeled and exposed to TG simultaneously (SIM protocol), extensive DPC production was observed (700-10000 rad eq.), indicating that lesions

involve both parental and daughter DNA strands. TGdR treatments gave similar results. DPC were dependent on dose and exposure time although the SIM protocol consistantly gave somewhat higher levels of DPC. DPC persisted for at least 24 h following drug removal. If cells were treated simultaneously with TG and hydroxyurea (1mM), DPC formation was almost completely prevented, suggesting a dependence on DNA synthesis and TG incorporation. Treatment of DNA with proteinase K (0.5 mg/ml in sarkosyl lysis solution) on the filters prior to elution reduced DPC by 85-95% in both DNA strands.

Protein crosslinking to DNA synthesized in the presence of TG is consistant with incorporation of the analog followed by formation of a disulfide or other covalent link between the thiol group of the anomalous base and nuclear protein(s). The observation of DPC involving the parental DNA strand is more difficult to explain, but may result from "trapping" of the template strand in a frozen replication complex. TG has been shown to cause chromosomal damage, particularlly when replication is attempted using a TG-containing template. The DPC lesions observed in our studies may play a significant role in the generation of this damage.

Proposed Course:

1. The effect of H_2-aza-C on DNA elongation will be further defined. The studies will include dose-response relationships and determination of the duration of the inhibitory effect. The relationship between inhibition of elongation, DNA SSB observed following prolonged drug exposure (24 h), and the characteristics of repair of these lesions will be explored.

2. The nature of TG-induced DPC will be investigated in greater detail. Thiol reducing agents will be used in an attempt to define the postulated covalent link between TG-containing DNA and protein.

3. The development of techniques to isolate proteins bound to DNA in the presence of TG will allow a determination of whether the lesion involves random nuclear proteins or results from an interaction with a specific protein involved in DNA replication.

4. The nature of parental strand lesions will be investigated by determining if SIM and SEQ protocol DPC demonstrate differential sensitivity to various methods of disruption (eg., minimal proteinase K digestion, digestion with other proteolytic emzymes, sensitivity to SDS containing elution buffers). If the parental strand is trapped in a non-covalent complex, such procedures may be expected to produce a differential reversal of DPC in the two strands of DNA.

5. It has been shown that maximal TG-induced SSB are observed when cells are labeled following TG treatment, at a time when the growing DNA strand is being synthesized on a TG-containing template. Experiments will be performed to see if DPC are also produced in this grandaughter DNA strand. Moveover, if the frequency of DPC in the TG-containing daughter strand correlates with the frequency of SSB observed in the grandaughter strand, this observation would provide a direct link between TG incorporation, DPC formation and the delayed chromosomal damage observed in TG treated cells.

Publications:

1. D'Incalci, M., Covey, J.M., Zaharko, D.S., and Kohn, K.W.: DNA alkali-labile sites induced by incorporation of 5-aza-2'deoxycytidine into DNA of mouse leukemia L1210 cells. Cancer Res. 45: 3197-3202, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06184-01 LMPH

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
RNA Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI:	William Bonner	Head, Chromosome Structure and Function Section	LMPH NCI
Others:	Christopher Hatch	Staff Fellow	LMPH NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION
Chromosome Structure and Function

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
1.2	0.8	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a set of procedures which simultaneously solubilize, denature, and stabilize the RNA present in whole cells or in cytoplasmic extracts for direct analysis by gel electrophoresis and hybridizaton of blotted gels. Multiple samples of tissue culture cells can be prepared for electrophoresis in less than an hour. The number of cells that can be processed for whole cell RNA is limited only by the sensitivity of detection of specific RNAs using hybridization probes. Cell preparations with high levels of RNase, such as human lymphocytes and HL60, can be processed reliably.

The method involves the stabilization of RNA in solubilized cells, cytoplasm or nuclei using various combinations of vandayl ribonucleoside complex (VRC), SDS, proteinase K, formaldehyde and heat, followed by resolution and specific detection of the RNA species on formaldehyde gels containing SDS.

Project Description

Introduction:

In many studies it is desirable to measure relative mRNA concentrations in cells subjected to various treatments. This generally involves the time consuming and not always reliable purification of RNA from multiple aliquots of cells even before any analysis can begin. The purified RNAs are then commonly analyzed by electrophoretic separation, blotting of the separated RNA species onto nitrocellulose or nylon supports, and hybridization of the blotted RNA with specific labeled probe. These procedures result in information about the sizes and amounts of the mRNA species of interest. As alternatives to purifying RNA, several dot blotting procedures have been developed which measure mRNA levels in unresolved cytoplasmic or whole cell extracts[3,4]; the disadvantage inherent in these procedures is the difficulty in separating the desired signal (specific hybrid formation) from the noise (background, non-specific hybrid formation or filter binding).

Objective:

1. To develop procedures for the direct analysis of RNA in cytoplasmic and whole cell extracts.

Major Findings:

1. The TurboBlot™ Processing System

In collaboration with American Bionetics, Inc., we tested a TurBlot™ processing system that improved the handling and processing of syntheses, Northern and dot blots. Significant savings in time and reagents were demonstrated. A paper describing this system was published.

2. Direct Analysis of RNA in Cytoplasmic and Whole Cell Extracts

We have developed a procedure in which many small samples of cells can quickly (in many cases in less than 1 hr) be prepared for direct electrophoretic analysis of intact whole cell or cytoplasmic RNA species. Attached cells are harvested by conventional or rapid techniques using RNase-free trypsin and serum or soybean trypsin inhibitor. RNA in the cytoplasm or solublized whole cells is first protected from degradtion by various combinations of vadadyl ribonucleoside complex (VRC)[5], sodium dodecyl sulfate (SDS), proteinase K, heat, and formaldehyde, and then the various species resolved in formaldehyde agarose gels containing SDS. After ethidium bromide staining, the RNA is transferred to hybridization membranes by existing capillary or electrophoretic techniques. The procedures are straightforward, require only small amounts of cells and allow the simultaneous analysis of multiple samples. The yield of RNA is quantitative and reproducible from duplicate aliquots of cells. Samples can be easily normalized to cell number or to the relative amount of 18 and 28s rRNA.

Significance:

The methodology just developed has extremely wide applicability in areas of cell biology in which information about gene expression is needed.

Proposed Course:

1. Testing the applicability of other procedures, originally developed for purified RNA, with RNA that has been stabilized and solubilized in whole cell or cytoplasmic extracts is certainly appropriate. In addition to isolating poly A containing RNA as discussed, we are also modifying methylmercury gels for use in the analysis of RNA in such extracts.

2. To adapt the methodology to utilize material from needle tissue biopsies to screen for expression of relevant genes, such as oncogenes.

Publications:

1. Compton, S.W., Stanchfield, J.E., Tabrizi, A., Wu, R.S., Mattern, M.R., and Galili, N.: The turboblot™ processing system: Improved southern/dot blot handling and analysis. BioTechniques 3: 308-312, 1985

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM-06185-01 LMPH

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 charecters or less. Title must fit on one line between the borders.)* Development of a Novel Assay for O^6-alkylguanine-DNA Alkyltransferase Activity in Cell or Tumor Extracts

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Roy S. Wu Expert LMPH NCI

Others: Kurt W. Kohn Lab Chief LMPH NCI

COOPERATING UNITS *(if any)*

American Bionetics, Inc., Emeryville, CA

LAB/BRANCH
Laboratory of Molecular Pharmacology, DTP, DCT, NCI

SECTION
Chromosome Structure and Function Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A sensitive assay for O^6-alkylguanine-DNA alkyltransferase activity in cell or tumor extracts has been devised. The theoretical basis of the new assay lies in the observation that certain restriction enzymes will not cleave DNA containing methylated bases. Thus if a synethetic oligodeoxynucleotide with a restriction sequence containing O^6-methylguanine were incubated with the restriction enzyme, this synthetic oligodeoxynucleotide should remain intact. However, if the guanine-O^6 methyl group were first removed by O^6-alkylguanine-DNA alkyltransferase present in certain cell or tissue extracts the synthetic oligodeoxynucleotide would be cleaved by the restriction enzyme.

The parental oligodeoxynucleotide and its restricton products are separated from each other and analyzed on denaturing polyacrylamide gels. The extent of cleavage by the restriction enzyme is a direct assay of the content of O^6-alkylguanine-DNA alkyltransferase in the cell/tumor extracts. The assay has been tested against cell culture and xenograft tumor systems and has performed in a predictive manner, correctly predicting all the Mer^- phenotype and 2 of 3 Mer^+ phenotype. Furthermore the assay is quantitative and the number of molecules of the O^6-alkylguanine-DNA akyltransferase per cell estimated using this assay agrees with those that have been published.

PHS 6040 (Rev. 1/84) GPO 914-918

Project Description:

Introduction

The DNA repair protein, O^6-alkylguanine-DNA alkyltransferase (GO^6AT), protects cells against mutagenic and cytotoxic effects of alkylating agents caused by alkylation of DNA at guanine-O^6 positions. GO^6AT levels in human tumor cell strains have been found to correlate with sensitivity to killing by chloroethylnitrosoureas (ClEtNUs). Approximately 25% of human tumor cell strains that have been tested show a phenotypic deficiency in GO^6AT and have been designated Mer^- Several of these GO^6AT deficient strains have enhanced sensitivity to killing by ClEtNUs, compared with strains having normal GO^6AT levels. This suggests that ClEtNU drugs may in principle be effective only in the treatment of GO^6AT deficient tumors. With the view that the rational clinical application of these drugs will require determination of GO^6AT levels in tumors in order to identify tumors that are potentially vulnerable to the drugs, we have devised a convenient assay that may be used for this purpose.

Objectives

1. Develop a simple and sensitive assay for GO^6AT
2. Determine levels of GO^6AT in tumors and relate the enzyme levels to the ability of cells derived from the tumors to form crosslinks in cell culture.
3. Collaborate with American Bionetics, Inc. to develop and produce this assay in kit form.

Methods

1. 5' end labeling of DNA with $^{32}PO_4$.
2. Labeling of DNA by terminal addition of ^{32}P-containing nucleotides (Klenow reaction).
3. Preparation of cell/tumor extracts.
4. Separation of nucleic acids from the macromolecules by column chromatography.
5. Restriction enzyme assay of synthetic oligonucleotide containing or not containing an abnormal base.
6. Separation of restriction enzyme products by electrophoresis or denaturing polyacrylamide gels.
7. Quantitation of restriction enzyme products by autoradiography.

Major Findings:

1. Development of a Denaturing Polyacrylamide Gel for Analyzing Small Oligonucleotides

A novel DNA sequencing gel different from that used in the Maxam Gilbert sequencing method was devised. The new DNA sequencing gel uses a discontinuous buffer system and is able to concentrate samples by its stacking action. Thus unlike the Maxam and Gilbert sequencing gel large volumes can be loaded without any loss of resolution.

2. Development of Assay for GO^{6}T

The development of a simple and sensitive assay for GO^{6}AT was undertaken. The theoretical basis of the assay lies in the observation that certain restriction enzymes will not cleave DNA containing either N^6-methyladenine, 5-methylcytidine or O^6-methylguanine in its restriction site. A deoxypolynucleotide was obtained from American Bionetics, Inc. containing O^6-methylguanine at a defined position in the sequence. The sequence was designed so that the O^6-methylguanine is at a restriction site which is blocked by the presence of the altered base. The assay for GO^{6}AT activity depends on the removal of the methyl group from the O^6 position which would then allow restriction endonuclease activity at this site. The parental oligonucleotide and its restriction products were separated from each other and analyzed on the denaturing polyacrylamide gels developed in this laboratory. The extent of cleavage by the restriction enzyme provided a direct assay for the content of GO^{6}AT in the cell/tumor extracts.

The following extracts were screened for their GO^{6}AT activity: cell extracts from IMR-90, VA13, HT29, BE, 1077, 1175, glioma (CLA), glioma (RIC) glioma (SAN); xenograft tumor extracts of HT29, BE, 1077 and 1175. The Mer phenotypes are known for 7 of 9 cell lines used in the assay. Only the Mer phenotypes of the 1175 and 1077 cells/xenograft tumors were unknown. The assay performed in a predictive manner, correctly predicting all the Mer$^-$ phenotype and 2 of 3 Mer$^+$ phenotype. Furthermore the assay was shown to be quantitative and the number of molecules of GO^{6}AT per cell estimated using this assay agreed with those that had been published. Currently, attempts are underway to link the oligonucleotide to a solid support (glass beads), the first step in the development of this assay in kit form. Several xenograft tumors (LK-1, MX-1, CX-1, and U-251), all in the NCI drug screening program, are being tested for GO^{6}AT activity.

Significance:

The studies of human GO^{6}AT aim to provide an assay by which tumor samples can be tested for potential sensitivity to treatment with chloroethylating drugs. In addition, an understanding of the origin of the GO^{6}AT deficit in some human tumor cells would lead to improved strategies for selective killing of potentially susceptibel tumors.

Proposed Course:

1. Continue development of assay in kit form.

2. Study possible correlations between GO^{6}AT activity, DNA crosslinking and the sensitivities of various human tumor cell types and of patients bearing the tumors to DNA crosslinking drugs.

ANNUAL REPORT OF THE LABORATORY OF TUMOR CELL BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1985 to September 30, 1986

The objectives of the Laboratory of Tumor Cell Biology are to develop, implement, and analyze data obtained from studies of cellular proliferation, cell differentiation, and biochemical growth characteristics of normal and malignant mammalian cells both in vivo and in vitro. Particular attention is given to hematopoietic cells, their normal behavior and especially changes seen during leukemogenesis. Because of unusual access to human blood cells and because of the interest of this group, there is special focus on human leukemias and lymphomas, and acquired immune deficiency syndrome. It is anticipated that an enhanced understanding of cell regulatory mechanisms will permit the optimal use of anti-tumor agents in the therapy of cancer and the development of new approaches.

The Laboratory of Tumor Cell Biology is concerned with several biological and biochemical problems: (1) Studies on the cellular and molecular origin and pathogenesis of human leukemia. Biochemical control mechanisms involved in cell differentiation and neoplastic transformation are examined. Tumor viruses of animals are used both as tools (to define and isolate genes and gene products important for growth in man) as well as for help in understanding mechanisms of naturally occurring animal leukemias and AIDS. Also, studies designed to determine the distribution of human T cell leukemia virus (HTLV) in T cell leukemia patients, patients with acquired immune deficiency syndrome (AIDS), and normals in different parts of the world are being carried out. (2) Studies on the biochemical events preceding mitosis. An understanding of these events appears essential to the control of proliferation, information derived from such studies may lead to more effective inhibitors of neoplastic cell growth. Events leading to mitosis are also of interest since many of the effective antitumor agents are useful only when cells are in DNA replication or in mitosis. Phytohemagglutinin stimulated human lymphocytes and tissue culture cells are the principal tools in these studies. (3) Attempts to develop new approaches to cancer chemotherapy using information gained from basic cellular studies. (4) Studies on the development of biochemical and immunological markers for malignant cells are carried out. Biochemical and immunological studies are also conducted in individuals with disorders associated with an increased incidence of neoplasia. (5) Controls regulating cellular growth and differentiation, and the process of malignant transformation in hematopoietic cells. (6) Growth factors (and their receptors) that control the growth and differentiation of blood cells have been isolated and are under intensive study, e.g., T-cell growth factor (TCGF), CSF, and related hematopoietic growth effecting molecules.

During the past year a number of findings were reported by investigators from the Laboratory.

Major Findings:

1. Isolation of HTLV-III from T-cells and cells of mononuclear/phagocyte series from the same patient.

2. Demonstration of biological effects of HTLV-III on monocyte/macrophage cells and long-term replication of HTLV-III in the infected cells.

3. Demonstration of selectivity of infection of monocyte/macrophage (MM) cells by HTLV-III grown on MM cells.

4. Development of endothelial cell lines from patients with Kaposi sarcoma and demonstration of the presence of neoangiogenic factor in these cells.

5. Demonstration of the presence of HTLV-III in tears, urine, and breast milk of AIDS patients.

6. Demonstration of increased prevalence of antibodies to HTLV-I (9%) and HTLV-II (18%) among intravenous drug abusers in New York, 41% of whom are also seropositive for HTLV-III.

7. Demonstration that HTLV-III envelope gene expressed by recombinant vaccinia virus induces antibody to the envelope protein in inoculated mice.

8. Demonstration by study of Japanese migrants to Hawaii that household transmission of HTLV-I at an early age is an important route of transmission.

9. Development of a human monoclonal antibody to the envelope protein of HTLV-I which may be important diagnostically and for the preparation of anti-idiotype vaccines.

10. In vitro immunoselection of an HTLV-III variant using neutralizing antibody positive serum, indicating that neutralizing antibodies can be type-specific and that they can influence the propagation of viral mutants.

11. Demonstration that pediatric AIDS cases who do better clinically and survive longer possess neutralizing antibodies while pediatric AIDS cases who do poor clinically or die lack neutralizing antibodies.

12. Demonstration that the protein products of the tat-III, sor, and 3'orf genes of HTLV-III are expressed in vivo and are immunogenic.

13. Development of second generation antibody tests for HTLV-III and procedures for standardization of HTLV-III serology.

14. Demonstration of D-Penicillamine and amphotericin analogs as inhibitors of HTLV-III replication in cell culture.

15. Demonstration of synthetic oligonucleotides complementary to the tat-III gene splice acceptor and donor sites as potent inhibitors of HTLV-III replication in cell culture.

16. Demonstration of the inactivation of HTLV-III by antibody to thymosin α-1 in cell cultures, indicating that antibody to thymosin α-1 is a neutralizing antibody.

17. Demonstration of the homology of thymosin α-1 to HTLV-III p17.

18. Preparation of monoclonal antibody to HTLV-III reverse transcriptase and demonstration of the precipitation of bands of 61,000 and 55,000 molecular weight by this antibody.

19. Determination of the nucleotide sequence of the envelope gene of five new isolates of HTLV-III and localization of conserved and divergent regions.

20. Localization of the tat gene on HTLV-III genome.

21. Demonstration that tat gene is essential for HTLV-III function.

22. Identification of proteins encoded by novel viral genes, sor and 3'orf, of HTLV-III.

23. Expression of HTLV-III gene products in prokaryotic systems.

24. Derivation of a noncytopathic replication competent mutant of HTLV-III.

25. Demonstrated nucleotide sequence homology between STLV-I and HTLV-I.

26. Developed a bovine-papilloma virus clone which expresses HTLV-I envelope proteins in mammalian cells.

27. Shown that HTLV-I infected leukemic cells do not undergo rearrangement for the beta chain of the T-cell receptor gene.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-06117-14 LTCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular and Physiological Control Mechanisms in Normal and Neoplastic Cells

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert C. Gallo	Chief	LTCB NCI
Marvin S. Reitz	Chemist	LTCB NCI
Prem S. Sarin	Chemist	LTCB NCI
W. Carl Saxinger	Microbiologist	LTCB NCI
Flossie Wong-Staal	Microbiologist	LTCB NCI

COOPERATING UNITS *(if eny)*

Rolf Neth, University of Hamburg; Robin Weiss, Imperial Cancer Research Fund, London, England; Dani Bolognesi and Bart Haynes, Duke University; Ken McCredie, M. D. Anderson Hospital and Tumor Institute

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION Sections on Hematopoietic Cellular Control Mechanisms, Hematopoietic Cell Biochemistry and Immunology, and Molecular Genetics of Hematopoietic Cells

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
56	33	23

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This Laboratory is concerned with five areas of research: (1) molecular and physiological control mechanisms in normal and neoplastic cells designed to obtain information on the molecular mechanisms involved in neoplastic transformation, including a search for and cloning of viral genomes and genome products in human tumor tissues; (2) the identification, isolation, and demonstration of biological activity of viral information in human leukemic cells and cells from patients with acquired immune deficiency syndrome (AIDS); (3) search for biochemical markers of minimal neoplastic disease and the development of practically useful microtests for the detection of such markers; (4) cell differentiation in vitro. (This relates to a major interest of the Laboratory: Does the phenotypic abnormality of leukemia in man result from a block in leukocyte maturation?) (5) Based on new information in the literature and from studies within this laboratory, new approaches to cancer chemotherapy are evaluated in in vitro and in vivo systems. This is the ultimate goal of the Laboratory.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

Suresh Arya	Cancer Expert	LTCB NCI
S. Zaki Salahuddin	Cancer Expert	LTCB NCI
Ann Sliski-Mark	Cancer Expert	LTCB NCI
Howard Streicher	Cancer Expert	LTCB NCI
Suzanne Gartner	Staff Fellow	LTCB NCI
Mary Harper	Staff Fellow	LTCB NCI
Marjorie Robert-Guroff	Staff Fellow	LTCB NCI
Rudolph Willis	Clinical Associate	LTCB NCI
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Anna Aldovini	Visiting Fellow	LTCB NCI
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Ruth Jarrett	Visiting Fellow	LTCB NCI
Takashi Okamoto	Visiting Fellow	LTCB NCI
Yoshitaka Taguchi	Visiting Fellow	LTCB NCI
Peter Biberfeld	Guest Researcher	LTCB NCI
Sandra Colombini	Guest Researcher	LTCB NCI
Amanda Fisher	Guest Researcher	LTCB NCI
Corrado Gurgo	Guest Researcher	LTCB NCI
Antoni Minassian	Guest Researcher	LTCB NCI
Megumi Nagata	Guest Researcher	LTCB NCI
Shuji Nakamura	Guest Researcher	LTCB NCI
Anne Marie Ranki	Guest Researcher	LTCB NCI
Reza Sadaie	Guest Researcher	LTCB NCI
Joseph Yourno	IPA	LTCB NCI

COOPERATING UNITS

M. D. Anderson Hospital and Tumor Institute; Myron Essex, Harvard University; Bill Haseltine, Harvard University; Jack Strominger, Harvard University; Roger Monier, Cancer Institute, Villejuif; Volker Erfle, Munich; Fernando deNoronha, Cornell University; Ivor Royston, University of California at San Diego; Bill Blattner, Epidemiology Branch, National Cancer Institute; Mark Smulson, Georgetown University; Isaac Witz, Tel Aviv University; Daniel Zagury, University Pierre et Marie Curie, Paris

Project Description:

Objectives

1. It is anticipated that a greater understanding of the processes involved in the molecular control of cellular growth, differentiation, and carcinogenic transformation, including the pathogenesis of human neoplasias and AIDS, will lead to the ultimate goal of developing improved approaches to therapy of human neoplasia. Special focus is on the leukemias, lymphomas, and AIDS.

2. The development of "markers" of neoplastic cells may lead to (a) quantitation of residual tumor cells after therapy and (b) determining whether cells (e.g., in leukemia) of patients in remission are really normal.

3. To develop new concepts of chemotherapy and apply them to animal model systems as rapidly as possible as new information is derived from basic experimental studies.

These objectives have primarily been pursued by the following approaches:

1. Biochemical studies on the properties of the RNA of type-C viruses and on the overall pathway of replication of these viruses. Purposes:

   a. To obtain more information on the mechanism of transcription of this RNA to DNA via reverse transcriptase.

   b. To determine if diagnostic probes can be obtained; i.e., is their structure specific enough that we can use this information to find viral RNA in cells by in situ hybridization?

   c. In understanding the mechanisms involved in integration and expression of viral genes, we can plan approaches to interfere with this expression and then evaluate the overall biological effect of this interference. We particularly wish to know if viral expression is required to maintain the cell in the neoplastic state.

2. Pursuing studies leading to an understanding of the origin of tumor viruses, how they acquire their oncogenic potential, how they interact with cells, and how they are transmitted throughout nature. These studies are primarily carried out with techniques of molecular hybridization, restriction enzyme analysis, and gene cloning.

3. Leukocyte differentiation in vitro. The soft agar technique for investigating maturation and proliferation of normal and leukemic human bone marrow cells was recently set up in our laboratory. Attempts are made to study exogenous and endogenous (released from feeder layers of normal cells) factors which affect these processes. Attempts have been made here and in other laboratories to differentiate human leukemic blast cells with apparent success. The implications of this to understanding leukemogenesis and for potential therapeutic approaches are obvious. The mechanisms involved in the maturation process are under study.

4. Growth of leukemic myeloblasts in liquid suspension under the stimulus of a conditioned media factor produced by human embryonic culture cells.

5. Markers: (a) Immuno-chemical technique for finding reverse transcriptase and other viral macromolecules in intact cells are being developed. (b) Techniques for detecting viral-specific nucleic acids in intact cells are also being developed.

6. Cell separation studies are being carried out to enrich subpopulation of leukemic cells which may contain the type-C RNA tumor virus-related markers and other biological markers.

7. Techniques are being developed to use monoclonal antibodies, prepared against cell surface antigens, for subtyping and separation of peripheral blood and bone marrow cells with the help of a fluorescence activated cell sorter.

9. Recombinant DNA technique is being utilized to obtain molecular DNA clones of defective and non-defective primate and human viruses. DNA from these clones will be utilized to carry out transfection experiments and for generation of subgenomic fragments for probes and functional analysis.

9. Human T-cell growth factor (TCGF) has been purified to homogeneity for further characterization. Studies are in progress to determine receptors on activated T-cells for TCGF.

10. The distribution of HTLV in human T-cell leukemia patients (HTLV-I, HTLV-II) and patients with acquired immune deficiency syndrome (AIDS) (HTLV-III) from various parts of the world is being actively pursued.

Methods Employed

1. Human leukocytes were isolated and purified as previously described (J. Clin. Invest. 48: 105-116, 1969; Science 165: 400-402, 1969). PHA stimulation of purified lymphocytes has also been described (Biological Effect on Polynucleotides, Springer-Verlag, New York, 1971, pp. 303-334; Blood 37: 282-292, 1971).

2. DNA polymerase activities were purified and characterized as reported (Nature New Biology 240: 67-72; Proc. Nat. Acad. Sci. 69: 2879-2884, 1972; Proc. Nat. Acad. Sci. 69: 3228-3232, 1972; DNA Synthesis in vitro, Proceedings of the Second Annual Steenbock Symposium, 1972).

3. Viral reverse transcriptase was purified and studied as described (Nature 234: 194-198, 1971; J. Virol. 12: 431-439, 1973; Biochem. Biophys. Acta 454: 212-221, 1976, 479: 198-206, 1977, 564: 235-245, 1979; Virology 112: 355-360, 1981).

4. Macromolecular synthesis, viability, mitosis in leukemic and normal cells and the effects of specific agents were evaluated as described before (J. Natl. Cancer Inst. 46: 789-795, 1971; Science 165: 400-402, 1969).

5. In vitro leukopoiesis is studied by the soft agar technique. In addition human myelogenous leukemic leukocytes are propagated in liquid suspension culture (Science 187: 350, 1975).

6. Induction of type-C virus from "non-producer" cells by iododeoxyuridine is carried out essentially as originally described by Rowe and colleagues. Infectious units, focus formation, and plaque assays for virus are carried out by conventional techniques.

7. Molecular cloning and molecular hybridization studies are carried out by conventional and by newly evolved techniques. These include: (a) filter technique with DNA; (b) filter techique with RNA covalently attached (Proc. Nat. Acad. Sci. 70: 3219-3224, 1973); (c) Cesium sulfate gradient analyses; (d) S_1 nuclease treatment; (e) RNA-DNA hybridization by competition analyses (Methods in Cancer Research, Vol. XI).

8. Tissue culture, virus production, cell viability estimates, cloning of cells are all carried out by standard techniques. Established procedures for titering infectious, leukemic viruses (XC test) and transforming sarcoma viruses (focus formation) are routinely performed. Also, virus neutralization procedures are performed by standard procedures.

9. Virus quantitation, virus specific molecules, metabolism of viral RNA and proteins are studied by conventional techniques.

10. Cell separation studies are carried out using fluorescence activated cell sorter (FACS), ficoll-hypaque gradients, sucrose density gradients, free flow electrophoresis, and centrifugal elutriation. (Lancet 1: 508-509, 1976).

Major Findings

1. Isolation of HTLV-III from T-cells and cells of mononuclear/phagocyte series from the same patient.

2. Demonstration of biological effects of HTLV-III on monocyte/macrophage cells and long-term replication of HTLV-III in the infected cells.

3. Demonstration of selectivity of infection of monocyte/macrophage (MM) cells by HTLV-III grown on MM cells.

4. Development of endothelial cell lines from patients with Kaposi sarcoma and demonstration of the presence of neoangiogenic factor in these cells.

5. Demonstration of the presence of HTLV-III in tears, urine, and breast milk of AIDS patients.

6. Demonstration of increased prevalence of antibodies to HTLV-I (9%) and HTLV-II (18%) among intravenous drug abusers in New York, 41% of whom are also seropositive for HTLV-III.

7. Demonstration that HTLV-III envelope gene expressed by recombinant vaccinia virus induces antibody to the envelope protein in inoculated mice.

8. Demonstration by study of Japanese migrants to Hawaii that household transmission of HTLV-I at an early age is an important route of transmission.

9. Development of a human monoclonal antibody to the envelope protein of HTLV-I which may be important diagnostically and for the preparation of anti-idiotype vaccines.

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26. Developed a bovine-papilloma virus clone which expresses HTLV-I envelope proteins in mammalian cells.

27. Shown that HTLV-I infected leukemic cells do not undergo rearrangement for the beta chain of the T-cell receptor gene.

Significance to Biomedical Research and the Program of the Institute

As outlined in the Objectives, these studies are designed to obtain fundamental information on molecular and physiological control mechanisms and the pathogenesis of neoplasia with the ultimate goal of developing new and improved approaches for anti-tumor therapy. In addition, some studies are designed to develop biochemical "markers" of neoplastic cells.

Proposed Course

As described above, some projects will terminate and others will continue to be actively pursued.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07148-03-LTCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular Biological Studies on T-Cell Malignancies and Lymphomas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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S. Zaki Salahuddin	Cancer Expert	LTCB NCI
Suzanne Gartner	Staff Fellow	LTCB NCI
Mikulas Popovic	Visiting Scientist	LTCB NCI
Paul Markovits	Guest Researcher	LTCB NCI
Yoshitaka Taguchi	Visiting Fellow	LTCB NCI
Shuji Nakamura	Guest Researcher	LTCB NCI
Antoni Minassian	Guest Researcher	LTCB NCI

COOPERATING UNITS *(if any)*

Robin Weiss, Imperial Cancer Research Fund, London, England; Bart Haynes, Duke University; Ken McCredie, M. D. Anderson Hospital and Tumor Institute; Myron Essex, Harvard University

LAB/BRANCH

Laboratory of Tumor Cell Biology

SECTION

Hematopoietic Cellular Control Mechanisms

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
15	8	7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The cell biology studies have been focused on the role of human T-lymphotropic retroviruses on human T-cell malignancies and acquired immune deficiency syndrome (AIDS). More than 100 isolates of HTLV-I have been obtained from patients with adult T-cell leukemia/lymphoma, and over 200 isolates have been obtained from patients with AIDS and ARC. HTLV-I has been shown to be a transforming virus whereas HTLV-III is cytopathic. Both HTLV-I and HTLV-III have specificity for OKT4 positive T helper cells. HTLV-III has been transmitted to a cell line which is productively infected with HTLV-III thus allowing the production of large quantities of virus for cell biology, molecular biological and biochemical studies, and the development of an ELISA test for screening of blood. HTLV-III has also been isolated from saliva, semen, and tears from AIDS or ARC patients. HTLV-III isolates obtained from different patients show some genetic variations in the envelope region. HTLV-III from a Haitian AIDS patient has been found to be the most divergent compared with other HTLV-III isolates. The virus probably attaches to the T cells through the OKT4 receptor since treatment of the virus with anti-T4 blocks infection of the recipient cells. Drugs that block HTLV-III replication are being tested in in vitro systems. Preliminary studies indicate foscarnet, AL 721, and D-penicillamine may be useful in treatment of patients with AIDS or ARC. Studies in chimpanzees indicate the development of antibodies against HTLV-III antigens and viremia in these animals on inoculation with HTLV-III. Vaccine studies indicate the development of neutralizing antibodies against the virus envelope, and more recently antibody against thymosin α-1 was found to block HTLV-III replication in cell culture. Thymosin α-1 has 50% homology with HTLV-III p17, suggesting the possible use of a synthetic peptide from the conserved region to be an effective vaccine for AIDS.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07149-03-LTCB

PERIOD COVERED

October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biological Studies on HTLV and Oncogenes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Flossie Wong-Staal	Microbiologist	LTCB NCI
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Laboratory of Tumor Cell Biology

SECTION

Molecular Genetics of Hematopoietic Cells

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22	15	7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies on human retroviruses and oncogenes have been pursued with particular emphasis on their role in human disease. Two subgroups of a human T-cell leukemia virus, designated HTLV-I and HTLV-II, have the unique capacity to transform human T cells in vitro, leading to cloned cell populations. Molecular cloning and comparative analysis of the genomes of HTLV-I and HTLV-II revealed sequence conservation throughout but particularly in a coding region designated tat and in enhancer sequences in the viral LTR. These results have direct relevance in the possible mechanism of transformation by these viruses. Recently HTLV-III has been postulated to be the etiologic agent of acquired immune deficiency syndrome. We have molecularly cloned and sequenced the HTLV-III genomes. A comparative analysis of the multiple HTLV-III isolates has shown divergence in the env gene. HTLV-III has been found to be similar to LAV or ARV and shows sequence homology to visna virus. HTLV-III infection has also been found in brain tissues of AIDS patients with encephalopathy. This was made possible by the recently developed in situ hybridization technique. It is now possible to express various HTLV-III proteins in prokaryotic systems and show transfection of biologically active HTLV-III DNA and demonstration of cytopathic effect.

PHS 6040 (Rev. 1/84) GPO 914-918

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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Takashi Okamoto	Visiting Fellow	LTCB NCI
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Corrado Gurgo	Guest Researcher	LTCB NCI
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COOPERATING UNITS

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 CM-07150-03-LTCB

PERIOD COVERED
October 1, 1985 to September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Seroepidemiological Studies on Human T-Lymphotropic Retroviruses

PRINCIPAL INVESTIGATOR *(List other professionel personnel below the Principal Investigator.) (Name, title, leboretory, and institute affiliation)*

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COOPERATING UNITS *(if eny)*
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LAB/BRANCH
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SECTION
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TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
8	4	4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The worldwide distribution of HTLV infection, the mechanism of its transmission, and its role in various types of T-cell malignancies and patients with acquired immune deficiency syndrome (AIDS) and ARC has been extensively studied. A highly sensitive ELISA technique has been developed and extensively used for detection of HTLV infection. Seroepidemiological studies on the distribution of HTLV-III show that 95% of the AIDS and ARC patients and approximately 45% of healthy homosexuals carry HTLV-III antibodies. High incidence (65%) of HTLV-III antibodies has also been found in sera of Ugandan children collected in 1972. Detection of HTLV-III antibodies in sexual partners of AIDS and ARC cases in New York suggest heterosexual transmission of HTLV-III. Other seroepidemiological studies show that populations at risk for development of AIDS include Canadian and Japanese hemophiliacs, Haitian immigrants to New Guinea, Zairians, Rwandese, and male prostitutes in Singapore. The sera from AIDS and ARC patients has been shown to precipitate HTLV-III envelope proteins of 41,000; 120,000; and 160,000 daltons. HTLV-III inoculations into chimpanzees show that these animals seroconvert and virus has been reisolated from their peripheral blood indicating infection of these animals. None of these animals has so far developed AIDS-like syndrome. Increased prevalence of HTLV-I (9%) and HTLV-II (18%) antibodies have been observed among IV drug users who are also positive for HTLV-III (41%). The recombinant vaccinia virus induced envelope protein in mice was found to give an antibody response. These results are important in our efforts to produce a vaccine for AIDS.

SUMMARY REPORT

ASSOCIATE DIRECTOR FOR THE CANCER THERAPY EVALUATION PROGRAM

DIVISION OF CANCER TREATMENT

NATIONAL CANCER INSTITUTE

October 1, 1985 - September 30, 1986

I. GENERAL ORGANIZATION

The Cancer Therapy Evaluation Program (CTEP) is responsible for the administration and coordination of the majority of the extramural clinical trials supported by DCT. These programs include the activities of the Clinical Cooperative Groups, the Phase I and Phase II new agent development contractors, and the holders of investigator-initiated grants (R01 and P01) relating to cancer treatment. Certain programs in developmental radiotherapy, such as high LET radiation, are administered in the Radiation Research Program. The Phase I development of biologic response modifiers is handled by the Biological Response Modifiers Program.

The Investigational Drug Branch (IDB) is responsible for sponsoring trials of new investigational drugs and of evaluating them for efficacy and toxicity. It does this by: 1) Coordinating and monitoring the trials of new agents developed by the DCT; 2) Planning with members of the Clinical Investigations Branch (see below) overall strategies for new agent studies in specific tumor types; 3) Regulating the distribution of investigational new drugs for which DCT is the sponsor; 4) Maintaining close contact and ongoing dialogue with the pharmaceutical industry in an attempt to ensure that new agent development proceeds in a coordinated way.

The Clinical Investigations Branch (CIB) is responsible for development and implementation of disease-oriented treatment strategies across the spectrum of human malignancies. In doing so, it provides management and oversight of the clinical cooperative group program. It manages the oncology and nutrition portfolios of R01 and P01 grants.

The Regulatory Affairs Branch (RAB) monitors the conduct of clinical trials performed in the NCI-supported clinical trials network. It also assures that clinical investigators using experimental agents are in compliance with federal regulations regarding the use of such agents. At the start of the clinical testing of each investigational agent, RAB obtains Investigational New Drug (NDA) exemption authorization from the Food and Drug Administration (FDA) and maintains close communication with FDA in all matters relating to experimental drug studies.

The Biometric Research Branch (BRB) provides statistical consultation to the other branches of CTEP, to the extramural and some intramural activities of other programs in DCT, and to the statistical centers of the clinical cooperative groups. It also carries on research in statistical methodology relating to cancer clinical trials.

The Office of the Associate Director (OAD) integrates the efforts of the Branches.

The process of protocol review is administered within the OAD by a central Protocol and Information Office (PIO) which is also the receipt point at NCI for all protocols entered into the PDQ system. The Program Analysis and Management Office (PAMO) has responsibility for the technical management of CTEP's grants and contracts and carries out analyses, as needed, of certain fiscal and administrative issues of particular interest to the program. The OAD is responsible for overall program supervision and budgetary allocation.

II. ORGANIZATIONAL AND PROFESSIONAL STAFF CHANGES

During the past year the Regulatory Affairs Branch was created from two sections formerly located within IDB: the Quality Assurance and Compliance Section (QACS) and the Drug Regulatory Affairs Section (DRAS). Dr. Dale Shoemaker was named Chief of RAB. Ms. Kim Regan, formerly head of PAMO, assumed the position of Executive Officer, Divison of Computer Research and Technology. Dr. Roy Wu joined CTEP as Program Director of the RO1-PO1 portfolio in CIB. Dr. Dennis Cain, formerly Chief of the Grants Review Branch, Division of Extramural Activities, became Special Assistant to the Associate Director. Dr. Peter O'Dwyer left IDB to assume a senior staff position at the Fox Chase Cancer Center, Philadelphia. Dr. Brenda Foster of IDB left for a position at the Royal Marsden Hospital, Sutton, Surrey, United Kingdom.

III. HIGHLIGHTS IN PROGRAM DEVELOPMENT

A. Development of New Agents

1. Initiation of extramural studies with Interleukin II (IL2) and Lymphokine-Activated Killer (LAK) cells. Following the exciting preliminary results of Rosenberg, CTEP coordinated the establishment of extramural trials to confirm and extend this work. Funding was provided to six institutions to study patients with renal cancer, malignant melanoma, and colorectal cancer. Accrual of patients began 4-5 months after publication of the original Surgery Branch findings and as of the time of this writing, the trial in renal carcinoma has completed accrual.

2. Introduction of six new agents into clinical trial. These include an anthrapyrazole derivative (NSC 349174), kymarabine (Ara-AC; NSC 281272), 4-ipomeanol (NSC-349438), chloroquinoxaline sulphonamide (NSC-339004), tumor necrosis factor (TNF), and a hematoporphyrin derivative (Photofrin II™; NSC-603062) that sensitizes tumor cells to light.

3. Drugs entering Phase II. During the past year Phase II trials were initiated with trimetrexate, taxol, pibenzimol, and the radiosensitizer, SR 2508.

4. Studies with Monoclonal Antibody, 17-1A. Following the demonstration of antitumor effect in early clinical trials conducted in the Clinical Oncology Program and at the University of Pennsylvania, expanded trials with this anti-adenocarcinoma antibody are in progress in pancreas cancer at the University of Nebraska, University of Alabama, and the Fox Chase Cancer Center. The antibody is being used alone and in combination with gamma-interferon and leukopheresed cells.

5. Findings of Particular Interest With Investigational Agents

a. LAK-IL2. Although these trials are currently too early for analysis, it is already clear that the six extramural centers are able to administer this difficult and potentially toxic program in conformance with the standards of the Surgery Branch. Toxicity in the extramural trials thus far has been within acceptable limits.

b. Deoxycoformycin. This is probably the most active agent against hairy cell leukemia producing over 90% responses with minimal toxicity in patients with good performance status. CTEP is organizing randomized trials of deoxycoformycin against interferon in both splenectomized and nonsplenectomized patients.

c. Interferon-alpha. This agent also shows very significant and clinically important activity against hairy cell leukemia, with many patients exhibiting improvement in the quality of life, reduced incidence of infections and elimination of a need for blood transfusions. While resistance to interferon has occurred in some patients, the incidence is very low, and it appears that interferon will be effective long-term therapy for this disease. This agent has recently received FDA approval for this indication.

d. Ifosfamide. Ifosfamide in combination with VP-16 and cisplatin has demonstrated activity in refractory testicular carcinoma. The drug is extremely active as a single agent in sarcomas, and an intergroup sarcoma randomized trial is planned of doxorubicin and DTIC with or without ifosfamide. Other trials are testing for a lack of cross-resistance with cyclosphosphamide in sarcomas.

e. Suramin. This agent inhibits retroviral reverse transcriptase and was placed in clinical trial for the treatment of AIDS. From April 1985 to April 1986, 97 patients with AIDS and related conditions were accrued to the multi-center Phase I suramin trials. Clinical, virologic, immuologic and pharmacokinetic data were collected and are being analyzed to determine the potential value of this drug.

6. Developmental Studies of Particular Interest with Investigational Agents.

a. HMBA. Two Phase I trials using the 5-day continuous infusion schedule have now been completed with metabolic acidosis and mental status changes the dose limiting toxicities. Two Phase I trials are currently evaluating the 10-day continuous infusion schedule, and the exploration of biological endpoints as markers of differentiation are an intrinsic part of these trials. A Phase II trial in preleukemia is planned.

b. Flavone Acetic Acid. Phase I studies on one-hour infusion Q3 weekly, and 24-hour continuous infusion Q3 weekly have begun. Preliminary toxicity and kinetic information support pharmacologically based aggressive escalation schema recommended by the Blood Level Working Group.

c. Tiazofurin. A study to explore the effect of dose/schedule on clinical efficacy and biochemical effects (e.g. TAD synthesis) was initiated in acute leukemia.

d. Trimetrexate. There is preliminary evidence of activity of this drug in colon cancer. Randomized scheduling studies (daily X 5 versus single dose Q2 weekly) are being organized in both colon cancer (SWOG) and in head and neck cancer. A randomized comparison of trimetrexate versus methotrexate in head and neck cancer (ECOG) will be activated in September.

e. Carboplatin. A comparative (cyclophosphamide + cisplatin versus cyclophosphamide + carboplatin) study in ovarian carcinoma is now underway in SWOG.

f. Dihydrolenperone. This is the first compound brought to clinical trial because of activity in the human tumor colony-forming assay. Phase I studies are now in progress.

g. AZQ. A new Phase I trial of AZQ using 24-hour continuous infusion schedule has been activated, based upon an improved therapeutic index seen in cellular pharmacology studies. This schedule may be used in a comparative randomized efficacy trial against BCNU as the frontline treatment of brain tumors.

7. Closing of IND's.

Four IND's were discontinued because of a lack of promising results or because of the availability of higher priority congeners (desmethylmisonidazole, immune interferon [Meloy], leukocyte interferon [Meloy], and streptonigrin).

8. Areas of Special Emphasis.

During the past year Requests for Applications (RFA) were issued in the areas of biochemical modulation and differentiating agents. Applications were reviewed; several received excellent priority scores. Funding of these applications is currently under consideration.

9. Liaison with European Drug Development.

During the past year major efforts continued to develop collaborative liaisons with the components of the EORTC and the Cancer Research Campaign in Great Britain. The goal is to create a two-way flow of drugs across the Atlantic with minimum time delay. This effort also seeks to reduce unnecessary duplication of trials that had occurred in the past because investigators often wished to (or needed to) reproduce the results of a reported study in their own country.

This renewed effort is a response to the increased efforts devoted to cancer drug development in Europe. NCI is interested in the possibility of bringing promising drugs into the U.S. after early clinical trials in Europe. In addition to promoting increasing attendance of American and European investigators at meetings of mutual interest, the DCT is supporting an increased level of monitoring for certain high priority Phase I trials in Europe.

Recently procedures have been established for the systematic review by DCT staff of European drug development activities. Currently CTEP is exploring with the Dupont Company the feasibility of entering one of its compounds, DUP 785, into limited Phase I trials in the U.S. following an extensive Phase I testing program in Europe.

10. Drug Cost Containment.

Cost containment has been achieved through the closer scrutiny of drug requests and by continuing to reduce the distribution of commercially available drugs. The procedures for ordering Erwinia Asparaginase, one of the most expensive drugs in the program, were changed so that drugs were requested for specific patients. This minimizes stocking of the drug and should result in a marked decrease in future distribution. In FY '85, Group C and Special Exceptions drug distribution has decreased by $73,840 (17%) and $103,437 (40%), respectively. These and other procedural changes have significantly reduced the drug budget. The FY '85 budget decreased by $637,396, nearly 15% from the previous fiscal year; this was achieved without compromise of the scientific goals of DCT.

B. Clinical Trials

Many noteworthy contributions are being made by the clinical cooperative groups across the full spectrum of human malignancy. The following represents a summary of selected areas of particular progress.

1. Colorectal Cancer

The NSABP has completed a trial (C01) of adjuvant therapy in 1200 colon cancer patients, comparing 5-FU/MeCCNU/VCR chemotherapy, BCG, and observation for patients with Dukes' B and C lesions. Average follow-up is 59 months. Approximately one-third of the patients have died. Current analysis suggests superior outcome in the group treated with chemotherapy. In a companion trial for rectal cancer (R01), the NSABP compared surgery plus combination chemotherapy or radiotherapy with surgery alone. More than 500 patients have been entered to date, with early results suggesting benefit in relapse-free survival for one of the three treatments. Accrual to this study continues as the data mature. NSABP has also opened a Phase III adjuvant trial of post-operative, seven day, 5-FU portal vein infusion versus surgery alone in March 1984 (C02). Five hundred patients have been randomized to date.

The North Central Cancer Treatment Group (NCCTG) recently reported the results of a trial of adjuvant treatment of rectal carcinoma in 200 stage B_2 and C patients (Proc. ASCO 5:318, 1986). Patients were randomized to radiation alone or radiation preceded and followed by chemotherapy. Median follow-up is 29 months. Preliminary results suggest a significant advantage in time to recurrence in the combined modality arm. In a trial of adjuvant treatment in 408 stage B_2 and C colon cancer patients (Proc. ASCO 5:316, 1986), patients were randomized to levamisole with or without 5FU versus observation. Both experimental arms show significantly improved time to progression and survival compared to the surgery alone arm. Based on this experience with

levamisole, a confirmatory intergroup Phase III trial in adjuvant treatment of colon cancer was started in 1985. Accrual of approximately 900 patients will be completed in 1986.

There is also progress in the developmental therapy of colorectal cancer. High doses of leucovorin appear to increase the therapeutic index of 5FU, improving the response rate. The combination of 5FU and cisplatin appears to have greater activity than 5FU alone in at least some studies. The combination of methotrexate and 5FU in an appropriate sequence appears more active than 5FU alone. If confirmed in comparative trials now in progress, these results will motivate a new generation of adjuvant studies.

2. Breast Cancer

NCI-sponsored investigators in Milan have shown that CMF chemotherapy as a post-surgical adjuvant benefits women with ER negative, node-negative breast cancer and primaries less than 5cm. At 24 months follow-up, 93% of the treated group and 50% of the control group remain free of evidence of tumor recurrence. Overall survival was 100% for the treated group and 75% of the control group. Further study is required, however, because of the small numbers of patients involved in the study and the relatively short follow-up time. Data from the same group in Milan suggest that the subset of ER-negative, node negative breast cancer patients who will benefit from adjuvant chemotherapy are those with a high thymidine labeling index in the primary tumor. This observation, if confirmed, has important implications both for future trials and for patient care in general. CALGB has reported that the addition of a second combination chemotherapy regimen following a standard course of adjuvant therapy produces superior disease-free survival in patients with node-positive, Stage II breast cancer.

During the past year an NIH-sponsored Consensus Development Conference emphasized the benefits of adjuvant therapy for several patient subsets. A statistical overview of all randomized adjuvant studies coordinated by Mr. Richard Peto of Oxford has provided additional evidence that adjuvant chemotherapy significantly decreases the probability of relapse and death from breast cancer.

3. Urologic cancer

The Prostate Intergroup Study comparing leuprolide plus placebo with leuprolide plus flutamide completed accrual of 617 patients by April 1986. The utility of complete androgen blockade in producing durable responses should be answered when the data from this study has matured. The testis intergroup adjuvant study of patients with resected Stage II nonseminomatous germ cell tumors continues to demonstrate the effectiveness of adjuvant chemotherapy with PVB or VAB in preventing relapse. Those patients who relapse after no adjuvant chemotherapy, however, are almost uniformly salvaged at relapse with the same chemotherapy although toxicity of chemotherapy at relapse is more severe. A regimen of reduced toxicity (VPV) has been reported by SWOG to produce equivalent results to PVB in patients with advanced Stage II or Stage III testicular cancer. The population of patients with poor risk germ cell tumors has been further defined by investigators at Memorial Sloan-Kettering Institute. Additionally, the less toxic combination of cisplatin

and VP-16 has produced equivalent results to VAB-6 in an early analysis by the same investigators.

Combination chemotherapy (MVAC) has yielded very high response rates in metastatic bladder cancer, with a significant percentage of complete remissions. These results pave the way for bladder cancer adjuvant trials now in the planning phase. Similarly, if Rosenberg's results with LAK + IL2 can be confirmed extramurally, adjuvant trials in renal cancer will be initiated.

4. Head and Neck Cancer

The activity of the cisplatin plus 5-FU combination in head and neck cancer is established. The platinum analogs, CHIP and CBDCA, apppear to retain the activity of the parent compound while reducing the toxicity. SWOG and NCCTG have mounted complementary, three arm studies in advanced head and neck cancer testing methotrexate as control versus cisplatin/5-FU, versus CHIP/5-FU (NCCTG) or CBDCA/5-FU (SWOG). The activities of the Head and Neck Intergroup are described below.

5. Brain Tumors

Interstitial irradiation for malignant tumors in the brain has been administered widely throughout the country. From center to center the isotopes, surgical techniques, and dosimetry are highly variable. The BTSG currently has a pilot trial to standardize within the group the stereotactic surgical placement and three dimensional, volumetric dosimetry of interstitial irradiation in preparation for launching a Phase III comparison of equal doses of external beam versus interstitial irradiation for malignant gliomas.

6. Adult Leukemia

A number of clinically important subsets of adult acute lymphoblastic leukemia (ALL) patients have been identified by the CALGB using newly developed monoclonal antibodies. In approximately 30% of morphologically and histochemically diagnosed ALL, the immunologic phenotype is ambiguous. Patients whose blasts carry myeloid antigens have a particularly poor prognosis. This may provide the basis for phenotype-directed clinical trials. The Leukemia Intergroup is developing a strategy for the prospective treatment of acute non-lymphocytic leukemia (ANLL) based on a number of risk factors identified by retrospective analysis. Failures during induction can be categorized into treatment failure (resistant disease) and treatment death (hypoplastic death). More intensive treatment is needed for the former group, less toxic treatment for the latter. A bone marrow examination on day 6 of therapy is a valuable discriminant. Other factors which enter the therapeutic equation include age, performance status, and serum albumin. Based on such factors, formulas have been derived to determine appropriate therapy. Prospective trials are planned to validate the retrospective findings.

7. Pediatric Leukemia

CCSG has reported the superiority of intensive therapy for children with previously untreated acute lymphoblastic leukemia and unfavorable prognostic

features. The 18 month event-free survival for standard therapy was 68%; for the 2 intensive experimental therapies, 90% and 87%. POG has demonstrated that infants with ALL (a group with significantly worse outcome) have extensive and bulky disease more often than do older children and are more often affected with a prognostically unfavorable phenotype of acute leukemia which expresses Ia-like antigens but is more often PAS^- and $CALLA^-$. These clinical and biological differences explain in part the observed poor response to treatment of infants with ALL.

8. Pediatric Lymphoma

Investigators at St. Judes' Children's Research Hospital demonstrated that use of VM-26 plus Ara-C with an otherwise conventional plan of acute lymphoblastic leukemia therapy is an effective approach to the treatment of advanced childhood lymphoma. Twenty-two of 23 (96%) evaluable patients with Stages III-IV achieved CR; with a median follow-up of 2 1/2 years only four have relapsed. Investigators at University of Wisconsin reported a regimen for poor-risk childhood non-lymphoblastic lymphoma patients which has produced a 90% event-free survival (18/20) at one year, results which are superior to any previously published trial.

9. Pediatric Solid Tumors

The Intergroup Rhabdomyosarcoma Study Group has reported a 73% relapse-free survival for patients who have localized residual disease after surgery (group III, IRS-II). The previous study, IRS-I, demonstrated 59% relapse-free survival at 3 years ($p < .002$). The major reason for improved outcome were the superior results in children with parameningeal disease who received cranial radiation and intrathecal chemotherapy under IRS-II.

The CCSG studied 89 patients with untreated primary neuroblastoma to determine the relation between the number of copies of the N-myc oncogene and survival without disease progression. Analysis revealed that amplification of N-myc was associated with the worst prognosis: the estimated progression-free survival at 18 months was 70%, 30% and 5% for patients whose tumors had 1, 3-10, or more than 10 N-myc copies, respectively. These results suggest that genomic amplification of N-myc may have a key role in determining the clinical behavior of neuroblastomas.

C. Coordination of Clinical Trials Strategies and Intergroup Studies

In the past year CTEP has emphasized to the oncology community the need for rigorous prioritization of research questions needing clinical trial. This need stems from the simple fact that the number of worthy research questions is greater than can be addressed by NCI's existing clinical trials network. The need to concentrate on the key issues in developmental therapy and to address them with trials of adequate size is evident. As a direct result of CTEP's advocacy, the amount of intergroup coordination and planning has vastly increased in comparison with past years. Much of this is as a direct result of CTEP sponsorship of planning meetings, but much is also occurring directly between (among) the cooperative groups as a consequence of CTEP's position. We summarize here selected examples of the new attitude now prevalent in the planning efforts of the cooperative groups.

1. The breast cancer committees of SWOG and ECOG have reached agreement on an extensive series of joint studies covering the whole range of clinical breast cancer presentations.

2. A number of institutions with interest and expertise in autologous bone marrow transplantation have agreed on common strategies for the development of combinations of multiple alkylating agents in high dose. Once a regimen with acceptable toxicity is developed, the aim is to test it in brest cancer as an adjuvant to surgical or chemotherapeutic cytoreduction in a multicenter intergroup fashion.

3. An initial meeting of brain tumor committee chairmen from several cooperative groups (BTSG, RTOG, SWOG, NCCTG) and cancer center investigators (UCSF, UMCC) was held in May 1986 to begin to address intergroup issues in brain tumor clinical trials. The main item discussed was the feasibility and design of an intergroup trial in low grade adult gliomas, a problem which is becoming increasingly apparent as diagnostic techniques improve. Also under consideration is an intergroup trial of regional versus systemic chemotherapy for brain tumors.

4. The Head and Neck Intergroup anticipates launching a Phase III trial in nasopharyngeal carcinoma in 1987. The final design is under discussion but will probably compare radiation with and without chemotherapy. The initial effort of the Head and Neck Intergroup is a randomized trial of post-operative radiation versus radiation plus chemotherapy for patients with completely resected head and neck cancer. The protocol, which opened to intergroup participation on January 1, 1985, is now averaging greater than ten patients per month and is approximately one-third completed.

5. A number of investigators interested in cutaneous T-cell lymphoma (CTCL) recently met to discuss a collaborative trial. An algorithm was developed to assess biological response modifiers alone, in combinations, and in combination with chemotherapy. Protocols are in development and an intergroup effort is expected within the next year.

6. Both alpha-interferon and deoxycoformycin (DCF) are highly active in hairy cell leukemia. Responses are observed in >75% of IFN-treated patients, and in almost 60% of DCF-treated patients. Either drug may obviate the need for splenectomy and therefore they will be compared in a Phase III trial in non-splenectomized patients. Participants will include CALGB, SWOG, ECOG, several cancer centers, the NCI-Canada, and possibly, the Australian Clinical Trials Group. Approximately 100 patients/yr will be accrued over the next 2-2 1/2 years.

7. For the treatment of diffuse large cell lymphoma, a number of regimens have been developed which take advantage of new drugs and strategies. Several of these are thought to be superior to CHOP. A large intergroup study now underway compares CHOP with MBACOD, ProMACE/CytaBom, and MACOP-B to determine if therapeutic advances have actually been made. Although this study originated in SWOG, ECOG is expected to complete its current study and join SWOG in

approximately a year. We also anticipate extensive intergroup collaboration in the studies of lymphoblastic lymphoma. Primary lymphoma of the central nervous system is uncommon, but increasing in prevalence as a manifestation of AIDS. The current standard treatment is surgery and/or radiation therapy, although neither has been adequately studied. A study involving the BTSG and the RTOG will determine the roles of chemotherapy and radiation.

In addition to these recent initiatives, continuing intergroup collaborations include:

1. Intergroup Testicular Study

   This is a collaboration among seven cooperative groups and four large institutions having an interest in testicular cancer. The protocol is a randomized controlled study of adjuvant chemotherapy of Stage II resectable testicular cancer and monitoring of Stage I testicular cancer.

   For Stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapse) versus surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to idenify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography.

2. Intergroup Rhabdomyosarcoma Study (see above)

3. Intergroup Prostate (see above)

4. Intergroup Colon Adjuvant (see above)

5. Intergroup Soft Tissue Sarcoma

   Patients with primary soft tissue sarcoma (STS) after definitive surgery and/or radiation therapy are randomized to either a high-dose intermittent doxorubicin adjuvant therapy or a control arm of no further therapy. Most major national and regional groups participate.

6. Intergroup Mesothelioma Study

   Patients with limited unilateral thoracic mesothelioma recieve combined surgery and radiation therapy and are randomized to receive adjuvant doxorubicin or a control arm. Several major national groups participate.

7. Intergroup Melanoma Study

   Patients with intermediate thickness primary melanoma are randomized to have an excision with a 2 cm versus 4 cm margin and to have elective lymph node dissection or not. All cooperative groups participate as well as the NCI of Canada and the Melanoma Clinic, Australia.

D. Clinical Trials Review

During the past year CTEP initiated the coordination of a detailed review of the clinical trials conducted in the cooperative group program. Its purpose was to evaluate, for selected disease sites, the quality of the concepts, design, conduct, analysis, and presentation of group trials in order that the weaknesses might be corrected and the strengths preserved. Thus far reviews have been completed for testis, non-small cell lung, bladder, prostate, and colorectal cancers. They have been conducted by teams of peers selected by CTEP staff. These assessments have in general confirmed the great strengths of the cooperative group program, but have also revealed significant problems in such areas as accrual rates (suboptimal in 35-80% of protocols), sample sizes (inadequate in 27-40% of protocols), and study design (inadequate in 25-84% of protocols). Upon completion of this analysis, these data will be a valuable background against which to take corrective action. They have already provided impetus for the new emphasis on intergroup studies and a more critical approach to protocol planning and review.

E. Clinical Trials Reorganization

Over the past year CTEP has engaged cooperative group investigators in an intensive dialog concerning the future of cancer clinical trials. As noted above, we have been particularly concerned with seeing to it that the cooperative group program ask and answer the key questions in therapy in a timely manner and in a fashion that provides the reliable definition of medically important treatment differences with reasonable power. We also wish to insure that the system has within it as much flexibility as possible for the shifting of resources when required by changing scientific priorities. Thus far, this dialog has been extremely productive and has led to important changes in the usual modus operandi of many cooperative groups (see above). We are continuing to explore modifications in the fiscal structure of the groups to permit increased flexibility. We are actively engaged in the formulation of guidelines for the conduct of intergroup studies, so that the setting up of these trials in the future may be made less arduous. We shall shortly establish common formats for the reporting of response and toxicity across the whole cooperative group program. We are drafting a comprehensive set of program guidelines for the cooperative group program, in order to clarify for peer reviewers and grant applicants what we expect of the clinical trials efforts. We are engaged in active discussions with the staff members of the Division of Extramural Activities and the Cancer Clinical Investigation Review Committee (CCIRC) concerning many aspects of the cooperative group review process, particularly in the matter of budgets, so that the dollar allocation for clinical trials can be managed more rationally. Over the next few months we anticipate substantial progress in all these areas.

F. NCI-Pan American Health Organization: Collaborative Cancer Treatment Research Program (PAHO:CCTRP)

For many years, the collaboration between U.S. and Latin American investigators in the development of clinical studies was a major goal of this program. Recent efforts by CTEP staff and PAHO investigators resulted in significant improvements in the functioning of the CCTRP. Unfortunately budgetary considerations have forced an overall re-evaluation of the relative priority of this effort within DCT programs; the result is that it has become necessary to phase out direct NCI

support of the CCTRP. CTEP plans to continue working with CCTRP investigators and will continue to supply experimental agents to CCTRP protocols of high merit.

G. Representation in International Activities

CTEP is responsible for providing clinical input into various international agreements adminstered by DCT. The PAHO Treatment Research Programs have already been mentioned, as have the increasingly close links with the European Organization for Research on the Treatment of Cancer (EORTC) which has had for several years a strong link with NCI. A member of the EORTC attends the Phase I Working Group meetings in Bethesda; CTEP staff is represented on the Protocol Review Committee of the EORTC. CTEP staff also provides clinical input into the bilateral agreements between the U.S. and France, Italy, Germany, and Japan. Major emphasis in these agreements thus far has been on new drug testing.

H. Analyses in Progress

In its role as coordinator and sponsor of most NCI-supported clinical trials of cancer therapies, CTEP has the important responsibility of assessing the suitability of current approaches and methodologies. Among the many questions currently under investigation by CTEP staff are:

1. How accurately do the findings in preclinical toxicology predict (a) a safe starting dose in Phase I trials, and (b) the pattern of organ toxicities seen in the clinic?

2. How accurately has the NCI's preclinical tumor panel predicted activity of new agents in human cancer?

3. What is appropriate methodology for evaluating chronic toxicity as a function of cummulative drug dose?

4. How can the predictiveness of multivariate survival models containing covariates be quantified?

5. What are appropriate trial designs for evaluating the activity and dose-response relationships of biological response modifiers?

6. CTEP staff has been involved in the development of new statistical designs that improve the efficiency of clinical trials. One class of designs that has been developed permits early termination of trials for which interim results are not promising. Another class of designs are focused on enhancing the effectiveness and breadth of small scale pilot studies. These designs permit efficient selection of the most promising dose of a biological response modifier or the most promising regimen of a set of experimental treatments. The selected treatment is then thoroughly evaluated.

I. Sponsorship of Meetings and Workshops

In addition to the usual meetings of the new drug development contractors and the Biochemical Modulation Advisory Group and the numerous working sessions with extramural investigators necessary to carry out the programs mentioned above, several other meetings were of particular interest:

1. Symposium on Overviews of Randomized Clinical Trials

This major symposium was organized and sponsored jointly by the Biometric Research Branch, CTEP, and by the Clinical Trials Branch of the National Heart, Lung, and Blood Institute. It brought together statisticians and clinical investigators from the fields of oncology and cardiovascular disease to discuss the controversial issues involved in "pooling" or coming to an overall assessment of the effectiveness of therapeutic interventions based on mulitple randomized clinical trials. Proceedings of this symposium will be published in a special issue of Statistics and Medicine.

2. Symposium on the Development of Antifols

This meeting in Tampa, Florida, organized by IDB staff, brought together clinical and preclinical scientists working in this area. Proceedings will be published in the NCI Monograph series.

IV. PUBLICATIONS

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BIOMETRIC RESEARCH BRANCH

1. STATISTICAL PLANNING AND REVIEW OF CTEP SPONSORED CLINICAL TRIALS

The BRB reviews all CTEP sponsored extramural clinical trials. The BRB attempts to ensure that such trials are statistically planned, conducted and reported in a sound and efficient manner. BRB staff collaborate with other CTEP and extramural investigators in developing initial plans and designs for clinical trials. Some areas that have received particular attention during the past year include: International trial to evaluate hyperthermic perfusion for patients with Stages I-II malignant melanoma, Phase II trials of interleukin 2 and lymphokine activated killer cells plus interleukin 2 for various malignant diseases, dose-response studies of gamma interferon for malignant melanoma, studies of deoxycoformycin and interferon for hairy cell leukemia, intergroup study of patients with primary brain tumors, studies of high dose chemotherapy and autologous bone marrow transplantation; and a study of *in vitro* chemosensitivity testing.

The BRB serves as liaison to extramural statistical centers. The BRB visits centers and organizes national and international meetings for statisticians involved in major extramural cancer clinical trials in order to improve statistical and data management procedures. A meeting of cooperative group statisticians was held in Montreal in May 1986 and an international meeting on overviews of clinical trial results was held in Bethesda in May 1986.

2. COLLABORATIVE CLINICAL RESEARCH

BRB staff collaborate in the following areas of clinical research. Principal clinical collaborators are listed in parentheses.

a. National clinical trials of the staging and treatment of early ovarian cancer (Dr. Robert Young).
b. Multi-institution randomized clinical trials of nutritional support in the treatment of patients with solid tumors (Dr. Daniel Nixon).
c. Multi-institution clinical trials of chemotherapy in advanced unresectable head and neck cancers (Dr. Mario Eisenberger).
d. Lung Cancer Study Group Clinical Trials (Dr. Carmack Holmes).
e. Multi-institution clinical trials of magnetic resonance imaging (Dr. Francis Ruzicka).
f. Intramural clinical trials of the Biological Response Modifier Program (Dr. Longo).
g. National Registry of colorectal cancer patients who have had resection of hepatic metastases. This registry contains information on 897 patients. Analyses have been completed identifying subsets of patients who do well or poorly after hepatic resection and describing sites of recurrence.
h. Development of staging systems for patients with non-Hodgkins' lymphomas. A data base of 1200 well staged patients collected in the International Classification Project has been updated during the past year. Analysis is being conducted to attempt to improve on the Ann Arbor staging system for such patients.

i. Overview of results with hexamethylmelamine in advanced ovarian cancer (Dr. Foster).
j. Comparison of histologic classification systems for rhabdomyosarcoma (Dr. Underleider).
k. Overview of results with low dose Ara-C in the treatment of adults with acute non-lymphocytic leukemia and myelodysplastic syndromes.
l. Evaluation of results in the treatment of 1900 patients in phase I trials to determine the extent to which phase II drug activity can be predicted (Dr. Hoth).
m. Overview of phase II results for all NCI sponsored drugs entering clinical trial since 1975 (Dr. Hoth).
n. Investigation of anemia of chronic disease in patients with skin disorders (Dr. Peck).

3. PRECLINICAL DRUG DISCOVERY

a. A thorough evaluation of the "tumor panel experiment" has been completed in collaboration with Drs. Hoth, Marsoni and members of the Developmental Therapeutics Program. This represents an evaluation of the murine tumor panel used for screening compounds since 1975.
b. A statistical design and method of analysis has been developed for the new disease-oriented non-clonogenic in vitro assay employing human tumor cell lines.
c. Methods for the evaluation of histologic specificity of anti-tumor pre-clinical activity have been developed for clonogenic and non-clonogenic assays.
d. A mathematical analysis of the efficiency that might be gained by "group testing" several compounds together has been performed. Optimal group size has been determined based on the level of activity desired and the a priori probability of activity for a compound. This analysis is being extended to other applications such as group testing in screening populations for pathogen exposure (e.g., AIDS).

4. INTERNATIONAL SYMPOSIUM ON OVERVIEWS OF RANDOMIZED CLINICAL TRIALS

This major symposium was organized and sponsored jointly by the Biometric Research Branch and by the Clinical Trials Branch of the National Heart, Lung and Blood Institute. It brought together statisticians and clinical investigators from the fields of oncology and cardiovascular disease to discuss the controversial issues involved in "pooling" or coming to an overall assessment of the effectiveness of therapeutic interventions based on multiple randomized clinical trials. It was a very successful and stimulating symposium. The proceedings, including discussion, will be published in Statistics in Medicine. In addition to organizing this symposium, the branch has evaluated the role that overviews should play in cancer therapeutics. Three presentations have been made at national and international meetings and one editorial has been written on this topic.

5. METHODOLOGICAL GUIDELINES FOR THE PUBLICATION OF REPORTS OF CANCER CLINICAL TRIALS

These guidelines were developed in collaboration with Dr. Robert Wittes and were adopted by the editorial board of Cancer Treatment Reports. They constitute nine specific recommendations to substantially improve the quality of clinical trial reports. During the past year these nine guidelines were adopted by Cancer, The Journal of Clinical Oncology and Cancer Clinical Trials.

6. COMPARATIVE STUDIES TO EVALUATE MAGNETIC RESONANCE IMAGING

The BRB is collaborating with the Diagnostic Imaging Branch (Dr. Francis Ruzicka, Chief) of the Radiation Research Program in the conduct of prospective multi-institution evaluations of MRI relative to CT scanning and other modalities in the diagnosis of brain neoplasms, liver metastases, musculoskeletal tumors, cervical myelopathies, lung cancer, uterine neoplasms, and congenital heart disease.

The BRB participates in the following ways:

a. As primary statistician in the design of the uterine neoplasm study in collaboration with Dr. Hedvig Hricak of UCSF and the design of the congenital heart disease study in collaboration with Dr. Charles Higgins of UCSF.
b. Extensive involvement in the planning and supervision of the NCI data management and statistical analysis contract.
c. Developed the organization and statistical design for the multi-institutional comparative reading of MRI versus CT images. Developed the design for a three-day session involving the reading of approximately 1200 images, for approximately 600 patients (for four protocols), by 20 radiologists. The design involved assuring that for each case the MRI and CT images would each be read twice by separate individuals from outside the acquisition center, and that potential systematic effects such as fatigue or reviewer biases would be distributed evenly between the modalities.
d. Chairman of the Statistical Advisory Group, which involved conducting two one-day meetings to finalize plans for the organization and conduct of the image review sessions and discuss issues involving analyses of the individual protocols.

7. NEW METHODOLOGY FOR EVALUATING CHRONIC TOXICITY AS A FUNCTION OF CUMULATIVE DOSE

Assessment of the relationship between acute toxicity and dose of an administered drug is usually straightforward. This is not the case for chronic toxicity such as irreversible cardiac or lung damage. Because some patients receive low doses due to early death and because extended survival may be necessary to observe clinical evidence of toxicity, the usual dose-toxicity analyses are biased. We have developed new statistical methodology for such problems. We have used these new methods to re-analyze a large data base of the Southwest Oncology Group concerning the relation between cardiotoxicity and cumulative dose of mitoxantrone. The new methodology also has broad applicability to studies of second malignancies and to epidemiologic investigations of occupational health.

8. CONFIDENCE INTERVALS FOR REPORTING THE RESULTS OF CLINICAL TRIALS

Increased use of confidence intervals in published reports could reduce misinterpretation of results by both authors and readers. A manuscript describing the use of confidence intervals with the types of data commonly encountered in clinical trials has been accepted by the Annals of Internal Medicine. An improved type of confidence interval for the difference in response rates has been developed and a report submitted to a statistical journal.

9. STATISTICAL COMPUTING

a. The branch received its MicroVax II computer and installed the UNIX operating system and APL interpreter. The system is very useful and cost effective for simulations and interactive calculations. A variety of useful programs have been written that assist in the clinical trial planning process. Software is being obtained to provide capabilities for graphically oriented exploratory data analysis.
b. The BRB has extended its range of projects funded under the Small Business Innovative Research Program. These projects are oriented to the development of computer systems for interactive analysis of data that bring state-of-the-art methodology and interactive analytical graphics to the disposal of statisticians, clinical investigators and bench scientists. The national response to this BRB sponsored research program has been substantial.

10. INTERGROUP STUDIES

a. With the input of CTEP staff and the extramural community of cancer cooperative group investigators, the BRB has developed a set of guidelines for the conduct of studies involving two or more cooperative groups. For scientific as well as financial reasons, it is expected that the number of intergroup studies will increase. In the past, intergroup studies have generally been developed and conducted in an informal manner. Many participants in intergroup studies have been frustrated by the lack of adequate quality control mechanisms, opportunities for input to study design, and regular monitoring reports. At the request of the cooperative group chairmen, and with input from the Clinical Investigations Branch, we have drafted a set of guidelines for the design and conduct of intergroup studies. With further input from cooperative group personnel (including statisticians, data managers, operations office staff, etc.), a final set of guidelines will be developed.

b. Facilitating the conduct of intergroup studies is an important priority of CTEP. The intergroup guidelines should contribute to this. The BRB is also working with group statisticians to determine, for several test intergroup studies, whether the amount of data collected can be substantially reduced without interfering with the quality or value of the research. Reduction in amount of data collection would both reduce costs and reduce the complexity of intergroup studies.

11. DESIGN OF PHASE II CLINICAL TRIALS

We have reviewed the statistical approaches to the design of single agent phase II trials, and have reviewed the response rates found in such trials for drugs

tested since 1975. Recommendations concerning the size, design and number of such trials for a drug have been developed. A manuscript has been submitted for publication.

12. QUANTIFYING PREDICTIVENESS OF MULTIVARIATE SURVIVAL MODELS CONTAINING COVARIATES

Identification of factors that predict the prognosis of cancer patients is important for advising patients, improving the efficiency of clinical trials and for more effectively formulating important therapeutic questions for appropriate subsets of patients. Many prognostic factors and multivariate models are "statistically significant" but not very predictive. We have developed methods to quantify the predictiveness of such models for predicting survival or disease-free survival. Such quantification is a useful step in the development of truly accurate predictors.

13. DEVELOPMENT OF DESIGNS TO EVALUATE ACTIVITY AND DOSE RESPONSE RELATIONSHIP FOR BIOLOGIC RESPONSE MODIFIERS

Phase II trials of biologics offer new challenges. One such challenge is that the appropriate dose for evaluating activity is unknown. Straightforward phase II trials at several doses permit the identification of whether the agent is active at any of the tested doses, but does not provide adequate precision to select a dose for phase III trials. We have developed and evaluated several designs for better characterizing the dose-response relationship or selecting a dose for phase III trials.

14. FOLLOW-UP TIME AS A MEASURE OF STUDY MATURITY

Median follow-up time is a commonly used measure of study maturity. This index is, however, ambiguously defined. Substantially different results can be obtained depending on how one deals with patients who have died or developed recurrent disease. An evaluation of the alternative definitions has been made and recommendations developed. A manuscript on this topic has been submitted for publication.

15. SUPPORT OF SURGICAL ONCOLOGY RESEARCH

In collaboration with the Clinical Investigations Branch, we are investigating trends in the submission and award of research grants to surgical oncologists. Variables studied include type of academic department, degree of principal investigator, type of grant and year of submission.

16. CONTINUING DEVELOPMENT OF THE CTEP INFORMATION SYSTEM (CTEP-IS)

The BRB collaborates with other CTEP staff in developing and evaluating the CTEP-IS. Particular issues of recent concern have been selection of data items, setting up cross-linkages with other information systems such as CLINPROT, and accuracy and consistency of coding.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 CM 06308-15 BRB

PERIOD COVERED
October 1, 1985 through September 30, 1986

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biometric Research Branch

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard M. Simon, Chief, Biometric Research Branch, CTEP, DCT, NCI
Others:
Susan S. Ellenberg, Statistician, BRB, CTEP, DCT, NCI
Lawrence V. Rubinstein, Statistician, BRB, CTEP, DCT, NCI

COOPERATING UNITS *(if any)* Developmental Therapeutics Program, DCT, NCI; Radiation Research Program, DCT, NCI; Biological Response Modifiers Program, DCT, NCI; Clinical Oncology Program, DCT, NCI; Environmental Epidemiology Branch, DCE, NCI.

LAB/BRANCH
Biometric Research Branch

SECTION

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20892

TOTAL MAN-YEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biometric Research Branch (BRB) is the statistical component for scientific planning and monitoring of the national and international research program of the Division of Cancer Treatment. The branch provides statistical leadership for all extramural activities of the division. The branch is also responsible for statistical consultation and collaboration with the intramural activities of the Biological Response Modifier Program, Developmental Therapeutics Program, and Radiation Research Program and performs collaborative research with components of the Clinical Oncology Program.

The Biometric Research Branch performs statistical planning and evaluation of all Division of Cancer Treatment supported therapeutic clinical trials. The branch performs scientific monitoring for the statistical aspects of the conduct and analysis of trials performed via cooperative agreement or contract. Primary statistical direction is provided by the branch for the conduct of selected national and international studies of therapeutic interventions, prognostic factors, pre-clinical screening and diagnostic imaging. The branch performs evaluations of therapeutic interventions based upon syntheses of results from multiple studies.

The Biometric Research Branch conducts research on experimental designs, biometric methods and biomathematical approaches for the development and efficient evaluation of improved cancer treatments.

PHS 6040 (Rev. 1/84) GPO 904-917

CLINICAL INVESTIGATIONS BRANCH

GENERAL BACKGROUND

The Clinical Investigations Branch (CIB) is charged with the responsibility for fostering and supporting the best possible extramural clinical oncology research. In performing this task, the CIB integrates its activities with all other Cancer Therapy Evaluation Program Branches and with selected NCI and NIH components. The generic methods employed by CIB include an integrated mixture of advisory, informational, and facilitative activities. The CIB attempts to be both active and reactive to promising scientific opportunities, that is, stimulating specific trials conceived intra- or extramurally.

COMPREHENSIVE DISEASE/MODALITY INFORMATION

In order to promote optimal studies, the CIB attempts to assemble a comprehensive disease and modality perspective for clinical research activities. In order to identify and articulate the most demanding research questions, the Cancer Therapy Evaluation Program makes use of diverse scientific information (including unpublished data from various academic and industrial sources worldwide). This information is a necessary, but not sufficient, ingredient for the generation of optimal studies. Additionally, CIB staff actively gather disease and modality information available to CIB staff--including interim data from domestic and foreign cooperative groups; information from scientific meetings; associated journal and editorial review responsibilities; the generation of scholarly publications and presentations by staff; and protocol and concept review by the Cancer Therapy Evaluation Program.

The staff of the Clinical Investigations Branch is organized such that specific responsibilities for modalities are as follows. Each individual is responsible for maintaining information on current and developing research opportunities and serves as an information resource to CTEP, NCI, NIH and extramural investigators as follows:

CIB MODALITY COORDINATORS

Modality	Staff
Bone Marrow Transplant	Cheson
Infectious Disease	Cheson
Nutrition	Stewart
Psychosocial	Stewart
Radiation	Hamilton
Surgery	Avis

Specific disease responsibilities are divided as follows:

DISEASE	STAFF
AIDS	CHESON
BRAIN	HAMILTON
BREAST	DORR
ENDOCRINE	NERENSTONE
GASTROINTESTINAL	HAMILTON

Specific disease responsibilities--Continued:

DISEASE	STAFF
GENITOURINARY	DORR
GYNECOLOGIC	STEWART
HEAD & NECK	HAMILTON
LEUKEMIA (ADULT)	CHESON
LUNG	STEWART
LYMPHOMA	CHESON
MELANOMA	STEWART
MYELOMA	CHESON
PEDIATRIC (LEUKEMIA + SOLID)	UNGERLEIDER
SARCOMA	STEWART

COORDINATION AND ADMINISTRATION OF THE COOPERATIVE GROUP SYSTEM

A major responsibility for the Clinical Investigations Branch is to advise and coordinate administrative and scientific aspects of the Clinical Cooperative Groups. This effort is necessary to optimize the productivity of a very powerful investigative instrument, the cooperative agreement mechanism (U10), through which the NCI provides funds for definitive (Phase III) multiinstitutional trials. A relatively large amount of money (approximately $50 million) is devoted to this mechanism. The CIB is responsible for and responsive to the Cooperative Groups; peer review judges the ultimate product. While the CIB has interest in related administrative and scientific aspects of the Groups, it is not concerned with their micromanagement. To be effective, however, all levels of the Cooperative Groups' structure potentially interact with the CIB staff.

The Clinical Investigations Branch advises and assists the Cooperative Groups in allocating scarce financial, physician and patient resources. During the past year, particular administrative problems have included: the reallocation of Type 5 budgets to provide interim funding for new institutional members to approved and funded Groups; to administer the phaseout of disapproved, unfunded Cooperative Groups; and to assist meritorious institutions from those disapproved Groups in finding new research bases within the Cooperative Group system. These activities were in addition to the more routine activities of devising and implementing a funding plan for successfully recompeting Groups, using available funds which represented a fraction of the amount recommended by peer review.

From a scientific point of view, the CIB and the Cooperative Group system identify and prioritize clinical research questions of interest. There is a potential interaction between the CIB and all Group organizational levels at any time in the protocol generation process. Specifically, CIB staff regularly attend formal Group meetings promoting idea stimulation and information exchange. An effort is made to prevent duplicative protocols and to foster the very best science.

A second area of interaction is through Concept Review, a relatively new activity for the CIB. Concept Review is an evaluation of the essence of a major Phase III study which is still in an early stage of development. A brief document outlining the scientific background, objectives, eligibility, treatment schema and statistical section are sent to the CIB, which provides relevant criticism in return. Theoretically, it is more efficient and productive to modify a concept

than to modify a protocol at the final stage of development. This format invites considerable and fruitful dialogue between the investigators and appropriate NCI representatives.

Thirdly, the formal Protocol Review process is in itself a major analytic activity. In this forum, a mature study plan that has already undergone considerable Group discussion and assessment is reviewed for safety and scientific issues. It is recognized that the Group has invested considerable energy into this protocol, and that it may be quite difficult to modify this document in any substantial way. There is, consequently, a continuum of CIB interactions with the Cooperative Group system from the very earliest idea formation to the review of the finished document.

Another exceedingly important area of interaction is the creation of a Cooperative Group Phase II/III strategic framework for disease or modality efforts. In order to most efficiently administer the Group resources, plans for orderly scientific development are highly desirable. Such planning provides an agenda of scientific issues. Obviously, for high interest, very promising, new areas of research, this plan would be modified. Also, the CIB encourages appropriate intergroup studies. Generally, at any particular moment, there are only a limited number of scientific questions of the highest priority. An intergroup study is deemed appropriate when a Cooperative Group study would require an inordinately long time for completion and/or might accrue too few patients to permit a powerful and complete statistical analysis. CIB staff consider it most prudent to be discriminating about the scientific experiments embarked upon and to attempt to rapidly answer selected questions with a large enough number of patients such that a medically meaningful statistical difference may be detected. Finally, the CIB has a responsibility to promote relevant laboratory--clinical correlative interactions which would prove mutually fruitful. Information concerning the best possible correlations comes not only from Group pilot activities, but also from information gained from the R01/P01 pool which CIB manages.

The following is a list of the Cooperative Groups which existed in 1985 and the appropriate CIB staff who were responsible for scientific liaison with that organization. Those Groups disapproved for further funding require staff assistance in implementing orderly phaseout plans.

GROUP	CIB STAFF
Brain Tumor Study Group (BTSG)	Hamilton
Cancer and Acute Leukemia Group B (CALGB)	Cheson
Children's Cancer Study Group (CCSG)	Ungerleider
Eastern Cooperative Oncology Group (ECOG)	Killen
Gastrointestinal Tumor Study Group (GTSG)	Hamilton
Gynecologic Oncology Group (GOG)	Stewart
Intergroup AML (IAML)	Cheson
Intergroup Melanoma Group (IMG)	Stewart
Intergroup Rhabdomyosarcoma Study (IRS)	Ungerleider
Intergroup Sarcoma Group (ISG)	Stewart
Lung Cancer Study Group (LCSG)	Stewart
Mid-Atlantic Oncology Program (MAOP)	Cheson
National Bladder Cancer Group (NBCG)	Dorr
National Prostatic Cancer Treatment Group (NPCTG)	Dorr
National Surgical Adjuvant Breast Project (NSABP)	Dorr

Cooperative Groups (1985)--Continued

GROUP	CIB STAFF
National Wilms' Tumor Study Group (NWTSG)	Ungerleider
North Central Cancer Treatment Group (NCCTG)	Stewart
Northern California Oncology Group (NCOG)	Killen
Pediatric Oncology Group (POG)	Ungerleider
Piedmont Oncology Association (POA)	Stewart
Quality Assurance Review Center (QARC)	Hamilton
Radiation Therapy Oncology Group (RTOG)	Hamilton
Radiologic Physics Center (RPC)	Hamilton
Southeastern Cancer Study Group (SECSG)	Cheson
Southwest Oncology Group (SWOG)	Cheson

OPTIMIZING CLINICAL TRIAL METHODOLOGY

A major CIB responsibility is to optimize clinical trial methodology in order to maximize study speed and accuracy and to minimize cost. Attention is specifically given to the management of the Cooperative Group program. Increasingly, stringent standards of scientific merit and efficiency are being applied in the review of clinical trials. Moreover, there is a broader appreciation of statistical methodology considerations which dictate which proposed studies are most appropriate. Over the past year, the CIB has been responsible for two major analytic projects to critically evaluate clinical trial methodology.

Clinical Trials Reviews

The purpose of the clinical trials review activity is to evaluate, for selected malignancies, the quality of clinical trials conducted by the Cooperative Groups based upon concept, design, conduct, analysis and presentation. This was an attempt to identify generic strength and weaknesses in the current Group system and to suggest strategies for capitalizing upon the strengths and correcting the weaknesses. The clinical trials review was a semi-independent critical assessment (in some ways quite similar to peer review) of a representative sample of the entire system. Its purpose was to assess qualiitative and subjective as well as quantitative aspects of Group performance. These reviews focused on protocols for specific diseases activated since January 1977, of any modality, involving a Phase III or combination Phase II effort. The reviewers were asked to critically evaluate materials from each protocol, to try to maintain an "historical perspective" in viewing activities from several years ago, and to minimize individual Group evaluation. The major effort here was to identify general and disease-specific strengths and weaknesses; it was not to evaluate a specific Group's performance. The following clinical trials' reviews were conducted from February 1985 to March 1986.

Clinical Trials Reviews

Date	Disease	Reviewers
2/12/85	Testis	Ozols, Williams, Bosl, Pistenma, Whitmore
6/6/85	NSCLC	Livingston, Natale, Ruckdeschel, O'Fallon, Pater, Fuks, Earl, McKneally, Kahan
10/28/85	Bladder	Lamm, Gibbons, Flanigan, Hannigan, Anderson, Shipley, Byhardt, Yagoda, Anderson, Torti
11/5/85	Prostate	Stephens, Trump, Garnick, Byar, Krisher, Smith, Williams, Leibel, Pilepich
3/10/86	Colorectal	Steele, Hohn, Tepper, Gunderson, Mayer, Levin, Macdonald, O'Connell, Rockette, Stablein, Flemming

Considerable analysis is proceeding from this effort, but the preliminary conclusions which can be drawn are as follows. None of the disease areas evaluated was problem free, but all had some strengths. Of the nearly 225 protocols evaluated in five clinical trials' reviews, the following generic problems could be identified:

1. Hypothesis generation was deficient in 25 to 54% of protocols.
2. Study design was inadequate in 25 to 84% of protocols.
3. Accrual was a problem in 35 to 80% of protocols.
4. Proper sample size was not planned for in 27 to 40% of the protocols.

It could be generally agreed upon that there were certain deficiencies in current Group methods for conducting clinical trials which deserve specific attention.

Group System Reevaluation

Simultaneously, and in a parallel effort based upon some of the findings of clinical trials review, a comprehensive evaluation of structural aspects of the Group system, consisting of a detailed analysis of its potential inefficiencies and inadequacies, was undertaken. This is a periodic exercise conducted by CTEP to constructively critique this system. This evaluation was predicated upon the belief that there is an enormous amount of valuable activity going on within the Cooperative Groups and that the Groups represent a major research instrument.

Nonetheless, extensive meetings both intramurally and with extramural investigators (Cooperative Group Chairmen and selective Group and Cancer Center representatives) attempted to characterize the strengths and weaknesses of the current system. A productive dialogue was established, and a number of suggestions made about how to improve the existing system. As a result of these activities, a subcommittee of the Board of Scientific Counselors of the Division of Cancer Treatment was selected which will work in concert with the Cancer Therapy Evaluation Program to formulate specific recommendations.

Strategy and Tactics Meetings

In implementing better clinical trials methodology, several activities were undertaken by the Clinical Investigations Branch. These included strategy meetings to help overview and prioritize national efforts in selected disease sites. Expert oncologist from the Cooperative Group and Cancer Centers system met at the National Cancer Institute to review ongoing clinical experiments and to identify short-term priorities for research. The format of these meetings was to review the ongoing Cooperative Group clinical trials along with current estimates of accrual and projections of when studies would be completed with discussion devoted to strategies for the next generation of clinical trials. Where appropriate, intergroup efforts were encouraged in order to achieve greater economy and statistical power. Meetings were held to discuss Stages I and II Adjuvant Breast Cancer, Stages III and IV aggressive high grade non-Hodgkins' lymphomas and cutaneous T-cell lymphomas. These meetings resulted in considerable exchange of information and capitalizing of collaborative research in all three areas.

A second series of meetings was also successfully instituted. These tactical meetings were focused efforts in which authorized representatives of Cooperative Groups met in Bethesda to implement selected research priorities which has been previously agreed upon. Rather specific intergroup studies were the usual product of these meetings. Examples include the intergroup prostate study examining total androgen deprivation with combined LHRH and anti-androgen therapy, the intergroup colon adjuvant study evaluating 5FU with or without levamisole, the hairy cell leukemia studies looking at interferon and deoxycoformycin, and a series of autologous bone marrow transplantation studies. These organizational efforts were successful largely because of the collegial nature of the interactions between intra- and extramural investigators.

MANAGING CLINICAL R01, P01 AND CONFERENCE GRANTS AND CONTRACTS

A fourth area of responsibility from CIB is the management of the clinical R01, P01 and conference grant and contract portfolio.

Grants

The purpose of the CIB grants management is to integrate relevant research information from all available sources and to manage efficiently and effectively a large, scientifically diverse and interesting grant pool. Currently, the CIB serves as program staff for 123 R01's, 34 P01's and one conference grant. There are a large number of important clinical and laboratory clinical projects within this group of grants.

Number of Active Grants in FY 86

Code	Clinical Treatment	Cancer & Nutrition	Surgical Oncology	Total
RO1/R23	85 (81/4)	14 (14/0)	11 (10/1)	110
R21	0	0	6	6
R35	2	0	0	2
R43/R44	1 (R44)	0	0	1
R13	4	0	0	4
TOTAL	92	14	7	123

The program project grant activity is an especially important and meaningful one since these are large, complicated and expensive research efforts. Listed below are the principal investigators and titles of the CTEP program project grant portfolio.

CTEP-Funded Program Project Grants (PO1) (FY 86)

Principal Investigator	Title
Bertino, Joseph	Clinical Pharmacology and Cancer Chemotherapy
Blume, Karl	Bone Marrow Transplantation for Hematologic Malignancies
Bortin, Mortimer	Collaborative Bone Marrow Transplantation Program
Clarkson, Bayard	Cancer Chemotherapy Program Project
Clarkson, Bayard	Human Hematopoietic Tumors Program Project
Creaven, Patrick	Clinical Biochemical Pharmacology in Ca. Therapeutics
Das Gupta, Tapas	Research Trends in Surgical Oncology
Dicke, Karel	Studies in Autologous Bone Marrow Transplantation
Frei, Emil	Solid Tumor Autologous Marrow Program
Frei, Emil	Clinical and Experimental Pharmacology
Gale, Robert	A Program in Bone Marrow Transplantation
Green, Alexander	Studies of Childhood Solid Tumors
Henderson, Edward	Cancer Clinical Research (grant expired 11/85)
Herbst, Arthur	UCCRC: Clinical-Lab, Studies in Gynecologic Cancer
Kersey, John	Pediatric Oncology/Marrow Transplantation Prog. Project
Laszlo, John	Clinical Cancer Research Program
McCredie, Kenneth	Human Leukemia Research Center Program
McGuire, William	Medical Oncology Program Project
Moertel, Charles	New Approaches to Treatment of Gastrointestinal Cancer
Morton, Donald L.	Surgery, Immunology and Immunotherapy of Human Cancer
Morton, Donald L.	New Approaches to Surgical Oncology
Murphy, Sharon	Leukemia Program Project
Nathan, David	A Program for Investigation of Childhood Tumors
O'Reilly, Richard	Transplantation of Lethal Congenital Immunodeficiencies

CTEP-Funded Program Project Grants (P01) (FY 86)--Continued

Principal Investigator	Title
Preisler, Harvey	Clinical & Biological Studies of the Myeloid
Rosenberg, Saul	Clinical & Laboratory Studies of the Malignant Lymphoma
Salmon, Sidney	Medical Oncology Program Project
Santos, George	Bone Marrow Transplantation in Human Disease
Schlossman, Stuart	Biology and Treatment of Human Leukemia and Lymphoma
Seigler, Hilliard	Diagnosis and New Therapeutics in Surgical Oncology
Storb, Ranier	Aplastic Anemia Center (transferred to HLBI)
Thomas, E. Donnall	Adult Leukemia Center
Tormey, Douglass	Biology and Experimental Therapy of Breast Cancer
Wilson, Charles	Program for Treatment of Malignant Brain Tumors

Contracts

Istituto Nazionale per la Studia e la Cura dei Tumori

A major effort in breast cancer has been conducted through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received worldwide attention. Studies testing the value of noncross-resistant drug regimens in the adjuvant setting are currently in progress, as is evaluation of adjuvant therapy in women with negative axillary nodes.

Extramural Clinical Trials Office (ECTO)--EMMES

This contract provides operations and administrative support for a number of CTEP supported extramural research efforts. The services provided include: assistance in protocol and forms design; patient randomization; quality control data; coordination of scientific activities of clinical investigators, statisticians and project officers; planning of meetings and preparation of agenda, minutes, reports, communications, and related administrative tasks. The contractor also provides analytical support to CTEP in evaluating data obtained from extramural clinical research resources.

Intergroup Testicular Study

This is a collaboration among seven Cooperative Groups and four large institutions having an interest in testicular cancer. The protocol is a randomized controlled study of adjuvant chemotherapy of Stage II resectable testicular cancer and monitoring of Stage I testicular cancer.

For Stage II the study compares the disease-free and overall survival for surgery alone (with combination chemotherapy for relapse) versus surgery plus early adjuvant chemotherapy. Stage I patients are registered and monitored to identify prognostic variables which may predict recurrence in this group. The protocol also includes important biologic studies such as histologic typing, serum marker studies, and studies of the accuracy of lymphangiograms, CT scans, and ultrasonography. Progress presentations have been made at various Cooperative Group meetings: CALGB, SEG, SWOG, and NCOG. This study is nearing completion, and full analyses are forthcoming.

NCI-Pan American Health Organization: Collaboration Cancer Treatment Research Program (PAHO:CCTRP)

The collaboration between U.S. and Latin American investigators in the development of clinical studies has been the major goal of this program. An extensive reevaluation of goals has resulted in financial termination of this contract.

SUMMARY

Over the past year the Clinical Investigations Branch has provided leadership in several areas. In an analysis of the Cooperative Group system, considerable energy has been devoted to identifying the strengths of the system and prospectively planning for improvements in its organizational structure. The clinical trials reviews and intensive discussions concerning possible group restructuring have been demanding exercises. Financial modeling to predict Groups' needs for the following years in order to ensure a consistent level of high grade research has been accomplished. Guidelines for the program management of the Group system and for intergroup studies are in the process of being prepared.

A second major area which has occupied the CIB has been the continuing effort to improve the quality of research questions. The strategy meetings, tactical meetings and concept reviews have all led to better quality scientific questions being articulated. Further efforts will be necessary to properly integrate laboratory-clinical correlation studies. As a general product of this effort to organize these activities, the Branch has been productive in peer review publications and program presentations.

The challenge for the upcoming year clearly springs from our present activities in FY86. The first of these is the Cooperative Group reevaluation and potential remodeling and restructuring. In order to achieve the most efficient and productive Group system, considerable CIB energy will continue to be expended in this effort. Secondly, the establishment of national scientific priorities will continue to be an area of responsibility. Groups of extramural investigators will be called in to the Cancer Therapy Evaluation Program in order to help set sensible goals which will be equitably arrived at, clearly communicated and efficiently achieved. This effort will not stifle innovation by the extramural community. Rather it is meant to provide a helpful priority framework for major Phase III investigations which consume such substantial effort and money. Obviously, pilot studies and Phase II studies will continue to be done as they are now, stimulated by Cancer Center or Cooperative Group activities. No attempt will be made to legislate scientific creativity. The only goal here is responsible management and disciplined science. Thirdly, new scientific initiatives must continue to be the challenge for the CIB. Important scientific hypothesis must be identified in a timely manner and properly addressed in a definitive way.

SPECIFIC PROGRAM ACCOMPLISHMENTS AND PLANS FOR FY87

The Clinical Investigations Branch is oriented toward the clinical study of disease and/or modality issues. The following are selected highlights of the current program and specific plans for the future.

PEDIATRICS

Accomplishments

Pediatric Leukemia

1. CCSG reported the superiority of intensive therapy for children with previously untreated acute lymphoblastic leukemia and unfavorable prognostic features. The 18 month event-free survival for standard therapy was 68%; for the 2 intensive experimental therapies, 90% and 87%.

2. POG demonstrated that infants with ALL (a group with significantly worse outcome) have extensive and bulky disease more often than do older children and are more often affected with a prognostically unfavorable phenotype of acute leukemia which expresses Ia-like antigens but is more often PAS^- and $CALLA^-$. These clinical and biological differences predict and explain in part the observed poor response to treatment of infants with ALL.

Pediatric Lymphoma

1. Investigators at St. Judes' Children's Research Hospital demonstrated that use of VM-26 plus Ara-C with an otherwise conventional plan of acute lymphoblastic leukemia therapy is an effective approach to the treatment of advanced childhood lymphoma. Twenty-two of 23 (96%) evaluable patients with Stages III-IV achieved CR; with a median follow-up of 2 1/2 years only four have relapsed.

2. Investigators at University of Wisconsin reported a regimen for poor-risk childhood non-lymphoblastic lymphoma patients which has produced a 90% event-free survival (18/20) at one year, results which are superior to any previously published trial.

Pediatric Solid Tumors

1. The Intergroup Rhabdomyosarcoma Study Group reported a 73% relapse-free survival for patients who have localized residual disease after surgery (group III, IRS-II). The previous study, IRS-I, demonstrated 59% relapse-free survival at 3 years ($p < .002$). The major reason for improved outcome were the superior results in children with parameningeal disease who received cranial radiation and intrathecal chemotherapy under IRS-II.

2. The Children's Cancer Study Group studied 89 patients with untreated primary neuroblastoma to determine the relation between the number of copies of the N-myc oncogene and survival without disease progression. Analysis revealed that amplification of N-myc was associated with the worst prognosis: the estimated progression-free survival at 18 months was 70%, 30% and 5% for patients whose tumors had 1, 3-10, or more than 10 N-myc copies, respectively. These results suggest that genomic amplification of N-myc may have a key role in determining the aggression of neuroblastomas.

Future Plans

Pediatric Leukemia

1. POG will continue to expand its lymphoblast classification effort with the addition of a central cytogenetics facility to perform detailed banding studies. This, in conjunction with cryopreserved cell banking, will allow detailed examination of cases in which chromosome abnormalities are detected.

2. CCSG will complete a series of trials in childhood A.L.L. addressing the following questions:

   a. Low risk A.L.L. - to identify a group of patients who require minimal therapy.

   b. Average risk - to compare survival and toxic complications randomizing patients to ± daunomycin in induction; ± cranial XRT in consolidation; and ± delayed intensification.

   c. High risk - to compare two aggressive therapies, one featuring delayed intensification (BFM), the other featuring continuous intensification (LSA_2L_2).

   d. Leukemia with lymphomatous features - to evaluate the need for XRT to bulk disease and for prophylactic cranial XRT.

Pediatric Lymphoma

1. CCSG will be conducting a randomized trial, in children with newly diagnosed advanced Hodgkins Disease, comparing a regimen of MOPP and ABVD given in an alternating fashion, with ABVD followed by low dose regional XRT. POG will randomize low-stage Hodgkin's disease patients to 3 cycles of MOPP-ABVD or to 2 cycles plus low dose involved field XRT. Both studies aim to reduce the potential for late effects of XRT by demonstrating equivalence for the chemotherapy arms.

Pediatric Solid Tumors

1. POG is planning to definitively evaluate, in a prospective randomized study, whether autologous bone marrow transplantation done in first remission for children with disseminated neuroblastoma offers superior disease-free survival duration over that attained in a similar group of patients treated with chemotherapy only. Alternatively, CCSG will explore the feasibility and benefit of autologous bone marrow transplant with *in vitro* purging of marrow in a similar patient population.

2. CCSG will conduct, in children with post-operative residual medulloblastoma, a randomized trial comparing:

   a. Standard radiotherapy followed by intensive drug therapy ("8 drugs in one day"), with

   b. Postoperative "8 drugs in one day" followed by XRT and continued "8 drugs in one day".

3. The Intergroup Rhabdomyosarcoma Study Group will supervise a study of the pathology classification of rhabdomyosarcoma. The purpose of this comprehensive study will be to develop a unified pathologic classification system that can be used internationally and that hopefully has prognostic significance. There are four different classification systems currently in use, and major controversy exists regarding their reproducibility and clinical utility. Approximately 800 cases registered on Intergroup Rhabdomyosarcoma Study II will be the subjects of pathologic study by a panel of proponents and external reviewers who will employ the four systems; subsequent analysis will focus on ease of reproducibility and prognostic significance of each system. Achievement of the goals of this study will be an important step forward worldwide in treating patients with rhabdomyosarcoma.

ADULT HEMATOLOGIC MALIGNANCIES (LEUKEMIA AND LYMPHOMA) AND AIDS

Accomplishments

Adult Leukemia

1. A number of clinically important subsets of adult acute lymphoblastic leukemia (ALL) patients has been identified by the CALGB using newly developed monoclonal antibodies. In approximately 30% of morphologically and histochemically diagnosed ALL, the immunologic phenotype is ambiguous. Patients whose blasts carry myeloid antigens have a particularly poor prognosis. This may provide the basis for phenotype directed clinical trials.

2. The Leukemia Intergroup is developing a strategy for the prospective treatment of acute non-lymphocytic leukemia (ANLL) based on a number of risk factors identified by retrospective analysis. Failures during induction can be categorized into treatment failure (resistant disease) and treatment death (hypoplastic death). More intensive treatment is needed for the former group, less toxic for the latter. A bone marrow examination on day 6 of therapy is a valuable discriminant. Other factors which enter their therapeutic equation include age, performance status, and serum albumin. Based on such factors, formulas have been derived to determine appropriate therapy. Prospective trials should be completed in the next 2-3 years to validate the retrospective findings.

3. A number of clinical and laboratory observations suggest that agents which induce cellular differentiation may be an alternative to cytotoxic therapy for ANLL and myelodysplastic syndrome. Low dose ara-C, retinoids, and gamma-interferon induce differentation of human leukemia cell lines. This can be evaluated *in vivo* by assessing oncogene expression: *c*-myc correlates with proliferation, *c*-fos with differentiation: patients are treated with a differentiating agent, or combinations of agents, and oncogene expression measured and correlated with clinical response.

Adult Malignant Lymphoma

1. The CHOP regimen represented a major advance in the treatment of diffuse large cell lymphoma. Over the last decade a number of regimens have been developed which take advantage of new drugs and strategies. Several of these are thought to be superior to CHOP. A large intergroup study was

organized and initiated and is now underway comparing CHOP with MBACOD, ProMACE/CytaBom, and MACOP-B to determine if therapeutic advances have actually been made. Although this study originated in SWOG, ECOG is expected to complete their current study and join SWOG in approximately a year.

2. Adult T-Cell Lymphoma (ATL) is a newly recognized HTLV-I related T-cell malignancy which clusters in geographical regions such as Jamaica and Japan. While notoriously unresponsive to conventional combination chemotherapy programs, case reports suggest a high level of activity for deoxycoformycin. Therefore, a pilot study has just been activated in Jamaica to evaluate DCF in ATL patients who have failed CHOP. Approximately 20 patients will be accrued to this study in the next year prior to considering a multi-agent, DCF-based regimen for induction therapy.

AIDS

1. From April, 1985 to April, 1986, 97 patients with AIDS and related conditions were accrued to multi-center Phase I Suramin trials. Clinical, virologic, immunologic and pharmacokinetic data were collected and are being analyzed to determine the potential valuee of this drug.

Bone Marrow Transplantation

1. Using high dose chemotherapy with combinations of alkylators requiring autologous marrow rescue, investigators from the Dana Farber Cancer Institute have reported exciting response rates in heavily pre-treated patients. For example, 90% of women with metastatic breast cancer achieve a response, 10-20% of which are CR's. Future directions include substitution of analogues to reduce toxicity and increase efficacy, and to develop disease-directed combinations in definitive studies.

2. GVHD is a major cause of morbidity and mortality following allogeneic bone marrow transplantation (BMT). A number of methods of T-cell depletion have been associated with poor engraftment. New methods developed by Dr. O'Reilly and co-workers have resulted in marked reduction in graft-versus-host-disease (GVHD) and excellent engraftment.

Future Plans

Adult Leukemia

1. Both alpha-interferon and deoxycoformycin (DCF) are highly active in Hairy Cell Leukemia. Responses are observed in >75% of IFN-treated patients, and in almost 60% of DCF-treated patients. Either drug may obviate the need for splenectomy and therefore they will be compared in a Phase III trial in non-splenectomized patients. Participants will include CALGB, SWOG, ECOG, several cancer centers, the NCI-Canada, and possibly, the Australian Clinical Trials Group. Approximately 100 patients per year will be accrued over the next 2 to 2 1/2 years.

2. High-dose ara-C (HiDAC) induces complete remission in 25-50% of patients who have failed conventional dose ara-C, suggesting a potential role for HiDAC in untreated patients. However, HiDAC is also associated with substantial

toxicity. SWOG will attempt to characterize the role of HiDAC by comparing it with standard dose ara-C in induction and consolidation. Accrual for this study is planned for two years.

Adult Malignant Lymphoma

1. A number of cutaneous T-cell lymphoma (CTCL) investigators recently met to discuss a collaborative trial. An algorithm was developed to assess biological response modifiers alone, in combinations, and in combination with chemotherapy. Protocols are in development and an intergroup effort is expected within the next year.

AIDS

1. NIAID/NCI have co-sponsored an RFP to establish approximately ten AIDS Treatment Evaluation Units. The function of these Units will be to perform Phase I trials of drugs designated of interest by the AIDS Drug Discovery Committee, and eventually to participate in Phase II and randomized Phase III trials. These units will provide the focus of NCI/NIAID Efforts in AIDS treatment. Approximately 2-3 new drugs will be tested during the first year.

MALIGNANT BRAIN TUMORS

Accomplishments

A. Interstitial Versus External Beam Radiation Therapy--Gliomas

Interstitial irradiation for malignant tumors in the brain has been administered widely throughout the country. From center to center the isotopes, surgical techniques, and dosimetry are highly variable. The BTSG currently has a pilot trial to standardize within the group the stereotactic surgical placement and three dimensional, volumetric dosimetry of interstitial irradiation in preparation for launching a Phase III comparison of equal doses of external beam versus interstitial irradiation for malignant gliomas.

B. Strategy Development

An initial meeting of Brain Tumor Committee chairmen from several Cooperative Groups (BTSG, RTOG, SWOG, NCCTG) and cancer center investigators (UCSF, UMCC) was held in May, 1986, to begin to address intergroup issues in brain tumor clinical trials. The main item discussed was the feasibility and design of an intergroup trial in low grade adult gliomas, a problem which is becoming increasingly apparent as diagnostic techniques improve. A draft of a protocol of post-operative radiation versus treatment delay until disease progression has circulated. Endpoints will be survival and the toxicity of treatment (mental function).

Future Plans

1. IG Low-Grade Glioma Trial

An intergroup low grade glioma trial with the participation of the BTSG, RTOG, SWOG and University of California, San Francisco will be activated in 1987. With recent improvements in diagnostic technology (e.g. MRI),

this disease entity is becoming increasingly important as are questions of optimal management. The expected accrual of approximately 400 patients will be achieved in three years with follow-up of up to 15 years anticipated.

2. IG Systemic Versus Intra-arterial Chemotherapy Trial

   Phase II experience with intra-arterial therapy for brain tumors has been widespread. An intergroup trial to define the relative advantage and toxicity of regional versus systemic therapy is under discussion in 1986 with a projected starting date in late 1987 or early 1988.

3. The current standard treatment of primary CNS lymphoma is surgery and/or radiation therapy. A study involving the BTSG and the RTOG will determine the roles of chemotherapy and radiation. Patient eligibility will not include patients with AIDS.

4. The first, informal meeting of multi-disciplinary, neuro-oncology investigators was held at ASCO 1986. The opportunity to meet and discuss with fellow investigators was felt to be highly useful by most of the participants. An effort will be made to arrange an annual strategy session of clinical investigators in CNS tumors. The goals for 1986 will be to discuss #1-3 above and to agree on parameters for evaluation in Phase II brain tumor trials.

HEAD AND NECK CANCER

Accomplishments

1. Platinum Analog Studies Launched through SWOG and NCCTG

   The activity of the cisplatin plus 5-FU combination in head and neck cancer is established. The platinum analogs, CHIP and CBDCA, apppear to retain the activity of the parent compound while reducing the toxicity. SWOG and NCCTG have mounted complementary, three arm studies in advanced head and neck cancer testing the control arm, methotrexate versus cisplatinum/5-FU, versus CHIP/5-FU (NCCTG) or CBDCA/5-FU (SWOG).

2. Intergroup Adjuvant Radiotherapy +/- Chemotherapy Ongoing

   The RTOG initiated a randomized trial of post-operative radiation versus radiation plus chemotherapy for patients with completely resected head and neck cancer. The protocol, which opened to intergroup participation on January 1, 1985, is designed to compare the true adjuvant roles for radiation and radiation plus cisplatinum/ 5-FU. Accrual, now averaging greater than ten patients per month, is approximately one-third completed.

Future Plans

1. IG Nasopharyngeal Trial

   The Head and Neck Intergroup is anticipating launching a phase III trial in nasopharyngeal carcinoma in 1987. The final design is under discussion but will probably compare radiation with or without chemotherapy for incompletely resected lesions.

BREAST CANCER

Accomplishments

1. NCI sponsored investigators in Milan, Italy randomized 89 women with ER negative, node-negative breast cancer and primaries less than 5cm in diameter to observation or combination chemotherapy with CMF for 12 cycles following definitive local-regional therapy for the primary. Patients were stratified by menopausal status and the thymidine labeling index of the tumor. At 24 months follow-up, 93% of the treated group and 50% of the control group remain free of evidence of tumor recurrence. Overall survival was 100% for the treated group and 75% of the control group. The results suggest that in general, ER negative, node-negative breast cancer patients are at high risk of early disease recurrence, and lend further evidence to suggest a role for adjuvant chemotherapy in stage I breast cancer. Further study is required, however, because of the small numbers of patients involved in the study and the relatively short follow-up time.

2. Data from the same group in Milan suggest that a specific subset of patients with ER-negative, node negative breast cancer who will benefit from adjuvant chemotherapy can be identified; they have a high thymidine labeling index in the primary tumor. This observation, if confirmed, has important implications both for future trials and for patient care in general.

3. The CALGB has reported that the addition of a second combination chemotherapy regimen following a standard course of adjuvant therapy produces superior disease-free survival in patients with node-positive, Stage II breast cancer.

4. The NCCTG reported the early results of a Phase III trial in postmenopausal women with node positive breast cancer. Combination chemotherapy with or without tamoxifen produces prolonged disease free survival relative to no adjuvant therapy. It is too early for a meaningful evaluation of the impact of this adjuvant therapy on overall patient survival.

Future Plans

1. A major study comparing preoperative (neoadjuvant) chemotherapy with standard post-operative chemotherapy in the treatment of Stage II breast cancer will likely be developed in the next six months.

2. Autologous bone marrow transplantation will be evaluated in locally advanced, poor prognosis patients with metastatic breast cancer.

UROLOGIC CANCER

Accomplishments

1. The testis intergroup adjuvant study of patients with resected Stage II non-seminomatous germ cell tumors was reported; it continues to demonstrate the effectiveness of adjuvant chemotherapy with PVB or VAB in preventing relapse. Those patients who relapse after no adjuvant chemotherapy, however, are almost uniformly salvaged at relapse with the same chemotherapy although toxicity of chemotherapy at relapse is more severe.

2. A regimen of reduced toxicity (VPV) has been reported by SWOG to produce equivalent results to PVB in patients with advanced Stage II or Stage III testicular cancer.

3. The population of patients with poor risk germ cell tumors has been further defined by investigators at Memorial Sloan Kettering Institute. Additionally, the less toxic combination of cisplatin and VP-16 has produced equivalent results to VAB-6 in an early analysis by the same investigators.

4. An intergroup study of RT versus BEP chemotherapy in patients with advanced Stage II testicular seminoma has recently been started and will take three to five years to complete. This study follows important leads suggesting that chemotherapy reduces distant recurrence compared to the standard of treatment, radiotherapy.

Future Plans

1. An adjuvant study of interleukin-2 plus lymphokine-activated killer cells in patients at high risk for recurrence of primary kidney cancer following nephrectomy will be initiated. One-half of the patients will be followed with no therapy while the other half will receive one course of IL-2 plus LAK. This study is a logical follow-up to the NCI-intramural work of Dr. S. Rosenberg.

2. A study of comparing adjuvant and/or neoadjuvant combination chemotherapy with standard therapy in patients with muscle-invading bladder cancer will be initiated. Both bladder salvage and overall survival will be the critical endpoints of interest.

3. The role of early cytoreduction followed by radiation therapy will be compared to radiotherapy alone in patients with Stages B_2 and C prostate cancer by the Radiation Therapy Oncology Group.

4. For patients with Stage C prostate cancer treated by total prostatectomy, the role of adjuvant radiotherapy will also be tested in the coming year.

GASTROINTESTINAL CANCERS

Accomplishments

1. Esophageal Cancer

   The RTOG has undertaken a Phase III comparison in localized esophageal cancer of radiation alone or with cisplatin plus 5FU. The study will test whether four courses of chemotherapy plus radiation control distant metastatic disease following potentially curative local therapy, that avoids the morbidity of surgery (standard therapy).

2. Pancreatic Cancer--MoAb 17-1A + Interferon

   The monoclonal antibody 17-1A has been identified as having cytocidal activity against various tumors of gastrointestinal origin. Phase I, dose finding trials are written, and Phase II trials are being developed with 17-1A treatment,

alone and in conjunction with interferon enhanced, monocyte-induced antibody dependent cellular cytotoxicity.

3. Colorectal Cancer

a. NSABP Colon C01

The NSABP has completed a trial of adjuvant therapy in colon cancer which accrued approximately 1200 patients between 1977-1984. It compared 5-FU/MeCCNU/VCR chemotherapy, BCG, and observation for patients with Dukes' B and C lesions. Average follow-up is 59 months. Approximately one-third of the patients have died. Preliminary data suggest benefit for chemotherapy, especially in some patient groups.

b. NSABP Rectal R01

In a companion trial for rectal cancer, the NSABP compared surgery plus combination chemotherapy or radiotherapy with surgery alone. More than 500 patients have been entered to date, with very early results suggesting benefit in relapse-free survival for one of the three treatments. Accrual to this study continues as the data mature.

c. NSABP Colon C02

A phase III adjuvant trial of post-operative, seven day, 5-FU portal vein infusion versus surgery alone opened in March 1984. Five hundred patients have been randomized. The data is maturing.

d. NCCTG Rectal

The NCCTG reported the results of a trial of adjuvant treatment of rectal carcinoma in 200 stage B_2 and C patients (Proc. ASCO 5:318, 1986). Patients were randomized to radiation alone or radiation preceded and followed by chemotherapy. Median follow-up is 29 months. Preliminary results suggest a significant advantage in time to recurrence in the combined modality arm.

e. NCCTG Colon

The NCCTG reported the results of a trial of adjuvant treatment in 408 stage B_2 and C colon cancer patients (Proc. ASCO 5:316, 1986). Patients were randomized to levamisole with or without 5FU versus observation. Both experimental arms show preliminary, significantly improved time to progression and survival compared to the surgery alone arm.

f. IG colon adjuvant Based on the NCCTG experience with the role of the immune modulator, levamisole, a confirmatory intergroup Phase III trial in adjuvant treatment of colon cancer was started in 1985. Accrual of approximately 900 patients will be completed in 1986.

Future Plans

1. Colorectal Cancer

   a. NCCTG Adjuvant Rectal

      The NCCTG will begin a seven year, adjuvant trial of sandwiched radiation therapy and 5-FU with or without MeCCNU for rectal carcinoma. A secondary question in the trial will compare radiation plus bolus versus continuous infusion 5-FU.

   b. IG Adjuvant Conference

      An international strategy conference on adjuvant therapy for colorectal cancer will be held in 1987.

2. Carcinoid/Islet Cell

   a. IG Islet Cell

      The NCCTG will organize an intergroup trial with SWOG and ECOG to the efficacy and toxicity of streptozotocin plus adriamycin versus chlorozotocin plus adriamycin for advanced islet cell tumors.

LUNG CANCER

Non-Small Cell Accomplishments

1. The Lung Cancer Study Group, in a series of trials examining the role of CAP chemotherapy, have shown that such treatment is of benefit in delaying recurrence and prolonging survival for patients with locally advanced (AJC regional stage III) tumors of non-squamous histology, who can first be rendered free of gross disease by surgery. These patients are at high risk of early relapse without such adjuvant therapy.

2. The Eastern Cooperative Oncology Group have examined their data from several Phase III comparative trials of four separate chemotherapy regimens for NSCLC, and have shown that initially promising response rates have not led to improved survival for many patients with advanced disease. Indeed, their regimen which resulted in the highest response rate was associated with the shortest survival, perhaps due to toxicities of the drugs used. They have concluded that no regimen can currently be recommended as "standard" therapy, particularly for patients with advanced tumors and poor performance status.

3. The Southwest Oncology Group have evaluated the effectiveness of combination chemotherapy with respect to initial performance status in patients with advanced (metastatic) malignancy at the time of diagnosis, and shown that beneficial effects from drug treatment are seen only in patients with good (grade 0-1) performance status before treatment. Hence, future combination regimens may be more accurately assessed if the study population is restricted to patients who are potential responders.

Future Plans

1. As a result of their comparative trials of different types of adjuvant therapy, the LCSG are opening a study comparing immediate post-surgical adjuvant CAP chemotherapy to observation for patients with completely resected NSCLC. A similar comparative trial is being performed by the North Central Cancer Treatment Group.

2. Based on results showing the effectiveness of cisplatin against NSCLC, and early evidence of enhanced activity in combination with 5-fluorouracil, the LCSG will conduct a trial using this "neo-adjuvant" chemotherapy in an effort to reduce tumor bulk in locally advanced disease patients, converting those who are currently inoperable to an operable, potentially curable, state.

3. Improvements in radiotherapy are an active area of investigation, including alterations in fractionation (high doses once weekly, low doses several times daily), combinations with radiosensitizing drugs (SR-2508, WR-2721, misonidazole), intraoperative brachytherapy, and neutrons rather than conventionally used photons. The RTOG, CALGB, and ECOG will be exploring these questions in several clinical trials.

3. The LCSG plan to investigate a number of promising new approaches to early diagnosis, accurate staging, and treatment of early stage disease. These include the use of indium-labeled monoclonal antibodies and their correlation with pathological findings at the time of surgery; hematoporphyrin derivatives with argon lasers for the diagnosis and treatment of carcinoma-in-situ; evaluation of the use of IL-2 to alter the natural killer activity of tumor infiltrating lymphocytes; and clinical-pathological correlative studies of newer techniques of imaging the thoracic contents.

Small Cell Accomplishments

1. The North Central Cancer Treatment Group announced results of a Phase III study evaluating the addition of etoposide to standard CAV combination chemotherapy, showing that it delays recurrence and may prolong survival.

2. The CALGB, SECSG and other multimodality cooperative groups have instituted clinical trials using alternating non-cross resistant chemotherapy combinations or chemotherapy/radiotherapy combinations. Preliminary observations have shown increased toxicity but have also suggested that improved response duration and survival may result, perhaps significant enough to warrant the worsened short term morbidity for some patients.

Future Plans

1. Having a variety of active drugs opens the possibility of exploring their combination in schedules that take advantage of non-overlapping toxicities. Results of preliminary laboratory and clinical work combining chemotherapy and radiotherapy by similar considerations clearly indicates a need for larger comparative trials that will define the proper use of these combined modalities. The CALGB have initiated a phase III study that will address some of these issues. Additional trials are needed to address others.

2. A large intergroup effort, involving the LCSG, ECOG and EORTC, is evaluating the value of surgical resection of residual tumor after response to initial chemotherapy.

3. Cisplatin and etoposide have been shown to have synergistic antitumor activity, and are as active together in SCLC as any known combination of multiple other agents. Newly developed understanding of their biochemical mechanisms of action in tumor cells has led to a preliminary study by the North Central Cancer Treatment Group, manipulating treatment schedules to capitalize on these understandings. The NCCTG will undertake a large phase III study based on these findings to define which theoretically optimal schedule results in best clinical outcome.

4. The development of analogs of cisplatin, with apparent incomplete cross-resistance and non-overlapping toxicities, has led to the clinical question of whether higher total doses of atomic platinum can be given safely and may result in improved tumor cell kill. The Piedmont Oncology Association wish to pursue this question in a large clinical trial.

5. The CALGB will continue a comparison of cisplatin with the platinum analog, carboplatin, each combined with etoposide.

MELANOMA

Accomplishments

1. Immune manipulation has become clearly established as a promising area of effective therapeutic intervention, particularly in those patients with very small amounts of residual tumor. Multiple trials are ongoing to further assess these promising leads as well as the potential of combining immunotherapy with pharmacologic agents such as cis-retinoic acid, the NSAIDs, cimetidine, etc.

2. In advanced disease, results with very high doses of alkylating agents, such as thioTEPA, followed by autologous bone marrow rescue have demonstrated that complete remissions can be obtained with drugs, although toxicity may be, at present, prohibitive. The Southwest Oncology Group are continuing investigations of this approach.

Future Plans

1. Of the interferons, the biological activity of gamma-IFN in an optimal dose and schedule seems substantially better than any others. Results of recently completed work suggest also that the surgical adjuvant setting may be the best in which to do further evaluations. A national, NCIsponsored randomized clinical trial will be conducted to do this. ECOG and NCCTG trials of alpha-IFN as adjuvant therapy in high-risk patients continue.

2. The selected population of patients who have melanoma confined to an extremity can be rendered disease-free by surgery, but those whose tumors are >1.5 cm thick are at high risk to recur and ultimately die of melanoma. Alkylating agents, hyperthermia, and isolation perfusion have been reported to reduce the rate of recurrence. Members of the North American Perfusion Group will conduct a randomized study of hyperthermic limb perfusion with L-PAM as

surgical adjuvant therapy for these patients, combining their results with those of EORTC and WHO investigators who are already engaged in this research.

3. Building on recent data from the National Cancer Institute of Canada, a joint NCIC-SWOG study is under consideration to define the efficacy and toxicity of levamisole as adjuvant therapy for melanoma.

4. The NCI will sponsor a number of extramural trials of IL-2 in advanced malignant melanoma, following the extraordinarily promising results seen in early trials conducted by the Surgery Branch.

GYNECOLOGIC MALIGNANCIES

Ovarian Cancer

Accomplishments

1. While a number of drugs have previously been shown to have significant antitumor activity in this disease, including some cures, their toxicity has been a major problem, particularly in what is often a relatively debilitated population. Comparative clinical trials have established that effective therapy does not necessarily mandate prohibitive toxicity. For instance, Gynecologic Oncology Group studies indicating that the drug combination cyclophosphamide/ cisplatin, given in adequate doses, is an effective regimen without the additional toxicity of doxorubicin, hexamethylmelamine, and other agents previously included in most chemotherapy combinations.

2. Studies of salvage therapy regimens, performed by GOG members, the NCI, and other institutions, have demonstrated that cisplatin in high doses is effective salvage therapy even in women who have relapsed after initial cisplatin treatment. These clinical studies plus retrospective dose-intensity analyses and laboratory evidence suggest strongly the existence of a dose-response relationship in epithelial ovarian cancer.

3. Institutional pilot studies evaluating the use of intraperitoneal agents, such as cisplatin, cytarabine, or interferons, have been promising. Cooperative group studies have been opened to further evaluate these results in a comparative manner, such as the SWOG-ECOG intergroup trial comparing intraperitoneal vs. conventional intravenous cisplatin.

4. SWOG and the POA have examined the use of in vitro drug testing with the human tumor stem cell assay to predict clinical responses in ovarian cancer patients, finding at least 40% accuracy for single agents, and 65+% accuracy for combination chemotherapy.

Future Plans

1. Surgical extirpation of all or most tumor has become established as prerequisite primary therapy for the successful treatment of ovarian cancer. It is also clear, however, that some form of surgical adjuvant therapy is needed in most cases if cure is to be achieved. Whether optimal adjuvant therapy will involve drugs, biological agents, radiation, or a combination of modalities has not yet been demonstrated; whether each modality is better used

regionally or systemically is also an issue of major interest and importance. A variety of trials are planned or recently approved to address components of this question, including intraperitoneal chemotherapy, gamma-IFN, or radioisotopes (compared to conventional systemic combination chemotherapy); a comparison of systemic chemotherapy to whole abdominal irradiation; and evaluation of treatment duration. These studies will be performed by the SWOG, ECOG, GOG, RTOG, and possibly others in both group and intergroup efforts.

2. Because of its intra-abdominal location and invidious growth pattern, ovarian cancer is difficult to monitor clinically, and tumor may not be detected when its bulk is very small and potentially most treatable. The recognition of ovarian cancer antigens detectable in the serum, such as MB 70K and CA 125, and their correlation with clinical disease may overcome this hurdle to assessing the adequacy of therapy, just as similar biomarkers have altered the treatment of germ cell tumors. Such clinico-pathologic correlation will be part of the treatment studies conducted by the GOG. Groups such as SWOG, NCCTG, and ECOG will be urged to include similar ancillary analyses in their planned trials.

3. Evidence from small pilot trials indicates that the platinum analogs, carboplatin and iproplatin, are at least partially non-cross resistant with cisplatin. They are known to have different toxicity patterns (including less nephrotoxicity and emetogenesis); their relative activity against ovarian cancer, compared to cisplatin, is not defined. Clinical trials to compare the agents will be done (e.g., SWOG and NCCTG comparing cyclophosphamide/cisplatin vs. cyclophosphamide/CBDCA). The possible combination of platinum analogs, capitalizing on non-overlapping toxicities, is also being explored (University of Wisconsin).

Cervix, Vulvar, Endometrial

Accomplishments

1. The cooperative group programs in this area have done basically Phase II screening of drug doses and schedules, without promising results. Smaller institutional studies have been slightly more encouraging, such as the RPMI work showing enhanced control of locally advanced disease using hydroxyurea for radiosensitization.

Future Plans

1. The GOG will be building on the RPMI experience, comparing the use of OH-urea as a radiosensitizer to standard post-surgical radiotherapy for control of regional and lymphatic metastases.

SARCOMAS

Accomplishments

1. The intergroup (SWOG, ECOG, CALGB, SECSG, NCOG, POA) surgical adjuvant trial in soft tissue sarcomas, comparing Adriamycin given immediately vs. delayed until recurrence, has not reached its accrual goals, but will be closed in light of findings from Scandanavian and UCLA studies showing no differences in survival between adjuvant Adriamycin vs. no therapy.

Future Plans

1. CALGB and SWOG will initiate a comparison of Adriamycin + DTIC vs. Adriamycin + DTIC + Ifosfamide for advanced soft tissue sarcomas.

2. Results of the above trial will determine the treatment arm for the next intergroup trial of adjuvant therapy for soft tissue tumors, comparing the better arm to control.

3. ECOG will perform a comparison of three regimens for advanced soft tissue sarcoma: Adriamycin vs. Adriamycin + Mitomycin + cisplatin vs. Adriamycin + Ifosfamide.

4. A major intergroup study is planned in osteogenic sarcoma, comparing "standard" six-drug therapy to a three-drug regimen, with a secondary evaluation of the value of surgical resection followed by beta-IFN for recurrences confined to the lungs.

SURGICAL ONCOLOGY

Accomplishments

1. In conjunction with the Division of Cancer Prevention and Control, a Surgical Oncology Training Grant under the T-32 mechanism has been developed. The program announcement has been published, and grants have been submitted as of January 1986.

2. The Surgery Section is studying the national research activities of surgical oncology as they relate to the NCI. The goal is to determine some of the generic strengths and weaknesses of this research area. This study will be the basis of a five year action plan for enhancing the national surgical oncology research effort.

3. The Surgery Section has held workshops on surgical quality control within the Cooperative Groups and targeted areas of research interest to surgeons in order to heighten surgical participation in clinical research.

Future Plans

1. The Surgery Section will continue to promote the Surgical Oncology Training Grant because of its pivotal role in the future development of surgical oncology.

2. The study of the national surgical oncology research activities will be completed, and a five year action plan formulated.

3. The Surgery Section will begin to work with the Surgical Committees of the Cooperative Groups in an effort to enhance surgical participation in clinical research.

4. Targeted workshops will continue to be held. The primary areas of emphasis will be the clinical testing of biologic response modifiers, surgical quality control and adjuvant studies.

STAFF PUBLICATIONS--Continued

Marsoni, S., Ungerleider, R.S., Hurson, S.B., Simon, R.G., and Hammershaimb, L.D.: Tolerance to antineoplastic agents in children and adults. Cancer Treat. Rep. 69: 1263-1269, 1985.

Pession, A., Vecchi, V., Zurlo, G., Masera, G., Marsoni, S., Madon, E., Zanesco, L., Mandelli, F., Massimo, L., Ceci, A., Burgio, R., Schiliro, G., Paolucci, G., and Ungerleider, R.: Treatment of childhood average risk acute lymphoblastic leukemia (A.L.L.): The experience of the Italian Association of Pediatric Hematology and Oncology (AIL-AIEOP 8202). Proc. ASCO 5: 163, 1986.

Perloff, M., Killen, J.Y., and Wittes, R.E.: Small cell bronchogenic carcinoma. Curr. Prob. in Cancer 10: 173-214, 1986.

Wittes, R.E., Friedman, M.A., and Simon, R: Some thoughts on the future of clinical trials in cancer. Cancer Treat. Rep. 70: 241-250, 1986.

STAFF PRESENTATIONS

Bruce D. Cheson, M.D.

Is the Use of Low Dose Ara-C Warranted in Acute Myelogenous Leukemia and Myeloblastic Syndromes? American Society of Clinical Oncology, May 5, 1986, Los Angeles, California.

Suramin Therapy in AIDS and Related Diseases: Initial Report of the U.S. Suramin Working Group. International Conference on AIDS, June 24, 1986, Paris, France.

CONFERENCES, WORKSHOPS, SEMINARS

Richard S. Ungerleider, M.D.

Rhabdomyosarcoma Histopathologic Classification Workshop, September 2-5, 1985, Columbus, Ohio.

Working Session on Rhabdomyosarcoma Histopathologic Classification, October 1, 1985, Venice, Italy.

CONFERENCES, WORKSHOPS, SEMINARS

Richard S. Ungerleider, M.D.--Continued

Pediatric Phase I Investigators Meeting, November 15, 1985, Bethesda, Maryland.

Working Session on Pediatric Hepatoma, March 20, 1986, Los Angeles, California.

INVESTIGATIONAL DRUG BRANCH

The Investigational Drug Branch has the mission of developing new investigational drugs by sponsoring clinical trials to evaluate their pharmacology, toxicities, and efficacy. It does this by: (1) managing and monitoring Phase I trials of new agents developed by the DCT, (2) developing and implementing a plan for Phase II trials in specific tumor types, and monitoring the results of these clinical trials, (3) developing and implementing in collaboration with the Clinical Investigations Branch of CTEP Phase III trials in selected areas of promising activity observed in Phase II, and (4) regulating the distribution of investigational new drugs.

The Investigational Drug Branch is now divided into three sections. Two medical sections, one for the cytotoxic agents and one for the biologic response modifiers which are concerned with the clinical aspects of the drug development process; the Drug Management and Authorization Section which regulates the distribution of investigational new drugs to all NCI sponsored investigators. The professional staff of the Branch includes eight physicians and two pharmacists.

Scientific Highlights and accomplishments:

1) IL2/LAK cell extramural trials. The initiation of trials in renal, melanoma, and colorectal cancer is a major new initiative. The studies will be completed by the end of this report year.

2) Deoxycoformycin has shown extraordinary activity in hairy cell leukemia.

3) Phase I trials began with an anthrapyrazole derivative, kymarabine (Ara-AC), Photofrin II™, ipomeanol, and chloroquinoxaline, and with Tumor Necrosis Factor, a biological.

4) Phase II trials began with trimetrexate, taxol, pibenzimol, and SR-2508.

Other highlights and accomplishments:

1) Creation of the BRMP-CTEP Working Group. This group is a joint planning/steering committee for all extramural clinical trials with biologicals. It provides tight integration between the two principal programs concerned with biological development.

2) Establishment of a grant program for clinical studies in biochemical modulation. An RFP was issued at the end of the last year to fund clinical trials intended to test drug combinations which are based on preclinical evidence of synergism and knowledge of mechanism of action at the biochemical level. The unique feature of these grants is that the clinical trials will examine tumor tissues to determine whether the drug interaction is occurring as predicted by the preclinical model.

7) Publication of an Investigator Handbook. This is a comprehensive description of all policies and procedures for physicians and institutions conducting clinical trials with NCI sponsored investigational drugs.

4) Sponsorship of a meeting on the development of antifols. A major meeting was organized by IDB staff during the year which brought together preclinical and clinical scientists working in this field. Its proceedings will be published in NCI Monographs.

5) Development of a standard reporting form for Phase II trials. This form will standardize the information reported to IDB/CTEP on Phase II trials from all sources.

6) Drug Costs. Further steps were taken to reduce drug costs, including: 1) decreasing the amount shipped as a starter supply to investigators, 2) developing a cost estimate for investigational drugs to be available for each protocol at the time of CTEP review, and 3) requiring that orders for Erwinia asparaginase be done on a patient by patient basis.

BIOLOGICS EVALUATION SECTION

Establishment of Extramural LAK Cell Studies. Funding was provided for six institutions to confirm the Surgery Branch observations that IL-2/LAK cell therapy produced objective responses in renal cell carcinoma, malignant melanoma, and colorectal carcinoma. A secondary objective will be to determine the sensitivity of other malignancies to IL-2/LAK therapy.

Accrual of patients began two months after awards were announced. Twenty-eight patients with renal cell carcinoma and seven patients with malignant melanoma have been treated with IL-2/LAK as of May 1986. Some responses have already been seen but most patients are too early to evaluate.

Alpha Interferon as Therapy for Hairy Cell Leukemia. Patients with Hairy Cell Leukemia who have been treated with alpha interferon for over two years continue to respond, with improvement in the quality of life, reduced incidence of infections and elimination of a need for blood transfusions. While resistance to interferon has occurred in some patients, the incidence is very low,and it appears that interferon will be effective long term therapy for this disease.

Deoxycoformycin is another agent with a high degree of activity of Hairy Cell Leukemia. CTEP has initiated discussions with investigators to conduct a large scale trial in previously untreated patients with Hairy Cell Leukmeia to compare the frequency, duration and completeness of responses following treatment with either dCF or alpha interferon.

Creation of the BRMP-CTEP Working Group. Members of the BRMP and CTEP have met regularly over the past year to discuss issues regarding the clinical development of biologicals, evaluate the scientific basis for new agents, review protocols submitted to either Program, and to design clinical studies to extend observations from the BRMP Preclinical Screening Laboratory.

Establishment of Phase I Trials with Tumor Necrosis Factor (TNF). Two centers are currently in the process of determining a maximum tolerated dose and defining the toxicities of TNF in cancer patients. The University of Wisconsin is administering TNF to patients on a thrice weekly schedule. Patients at the Mayo Clinic receive TNF daily for 5 days, every three weeks.

Evaluation of the Monoclonal Antibody 17-1A in Patients with Colon and Pancreatic Cancer. Four centers are testing the efficacy of 17-1A in patients with gastrointestinal malignancies. The NCI-Clinical Oncology Program has treated patients with pancreatic cancer; several partial responses have been observed. Trials are in progress at the University of Nebraska, University of Alabama, and the Fox Chase Cancer Center. Some centers are studying 17-1A in combination with gamma-interferon or with leukopheresed cells.

DEVELOPMENTAL CHEMOTHERAPY SECTION

For FY'86, five new compounds will be brought into clinical trials, as follows:

1. Anthrapyrazole Derivative (NSC-349174)

   This compound is one of a series synthesized by the Warner-Lambert Company in a congener program to modify the anthracenedione nucleus in an effort to reduce the tendency to semiquinone free-radical formation. Long-term survivors were observed in antitumor studies in mice against the i.p. implanted B16 melanoma, M5076 sarcoma, and L1210 and P388 leukemia. Good activity was also demonstrated against the s.c. implanted L1210 leukemia and the human MX-1 mammary tumor xenograft. In studies done by the Warner-Lambert Company, good activity was observed against the s.c. implanted Ridgway osteogenic sarcoma, mammary 13C carcinoma, mammary 16C carcinoma, and colon 11A carcinoma. In biological characterization studies, the compound was shown to be a potent intercalating agent, to produce DNA strand breaks and to have a selective effect on DNA synthesis.

2. Kymarabine (Ara-AC) (NSC-281272)

   This compound is structurally an analog of both Cytosine Arabinoside and 5-Azacytidine combining the sugar of Cytosine Arabinoside with the base of 5-Azacytidine. Kymarabine demonstrated good antitumor activity against the CX-1 colon, MX-1 mammary, and LX-1 lung human tumor xenograft implanted under the renal capsule of athymic mice. The compound also demonstrated activity against the i.p. implanted L1210 and P388 leukemias and the i.v. implanted Lewis lung carcinoma. Kymarabine is metabolized to the monophosphate by cytidine kinase and is incorporated into DNA. However, preliminary studies indicate that incorporation into DNA and sensitivity of the tumor do not appear to correlate. Kymarabine is not deaminated extensively in P388 leukemia cells, but is deaminated in the kidney.

3. Ipomeanol (NSC-349438)

This compound is a cytotoxic furan derivative originally isolated from mold-damaged sweet potatoes. Ipromeanol is metabolized by pulmonary cytochrome P-450 monoxygenases to a reactive eletrophile(s) which binds predominantly in the pulmonary nonciliated bronchiolar cells (Clara cells). The compound was negative in the mouse tumor screen as would be expected for a compound which is activated by Clara cells. Activity has been observed in the human tumor colony forming assay in lung tumors with Clara cells. Therefore, the clinical trials will be directed toward lung tumors with Clara cells.

4. Chloroquinoxaline Sulphonamide (NSC-339004)

NSC-339004 is the second compound with activity in the human tumor colony forming assay (HTCFA) to be presented to the Decision Network Committee. The compound has outstanding activity in the HTCFA. The response rate at 10 g/ml was 29% (13/45) with an IC_{70} range of <0.1 to >100 g/ml. The rat hepatocyte co-culture had no significant effect on the HTCFA activity. In the P388 colony forming assay the IC_{70} was 55 g/ml. In addition, there was in vivo activity against the P388 and the CD8F1 murine mammary tumor (single active test in the CD8F1 tumor). Activity ws observed against 3/11 melanoma, 6/14/ ovary, and 4/8 lung tumors. Therefore, the following points summarized the rationale for the development of NSC-339004:

1. Outstanding activity in the HTCFA;

2. Superior in vitro activity against human tumor cells vs. mouse P388 cells;

3. In vivo activity against P388 and CD8F1 murine mammary tumor (single active test in CD8F1); and

4. The compound is likely to pass toxicology as a closely related compound was developed by MErck as an antibacterial drug.

5. Photofrin II™ (NSC-603062)

Photofrin II™ (dihematoporphyrin ethers DHE) is a concentrated form of the active ingredients of hematoporphyrin derivative. Photofrin II™ is a photo-sensitizing drug, which has been combined with activating light (630 MM) to treat a variety of localized tumors in animals ("Photodynamic therapy"). This therapy is based on the combination of properties of the photosensitizer; it is relatively non-toxic (except for a temporary generalized photosensitivity), is photochemically active, can be activated inside you by penetrated visable light, and is retained in nearly all malignant and some pre-malignant lesions to a higher degree and longer than in the normal surrounding tissues. Original clinical trials in 1978 and 1979 were conducted with Photofrin derivative; whereas some efficacy were seen in these trials, the selection of light dose were rather crude, and moderate damage to tissues were seen. The reinduction of this methodology in 1986 involves a more selective and penetrating light source, the removal of several known lower molecular weight less selective Photofrins from the activating drug and better defined clinical trials methodologies.

6. Flavone Acetic Acid

Phase I studies on one hour infusion Q3 weekly, and 24 hour continuous infusion Q3 weekly have begun. Preliminary toxicity and kinetic information support pharamcologically based aggressive escalation schema recommended by the Blood Level Working Group.

In addition to the Phase I drugs just mentioned our progress continues in the following areas:

1. Deoxycoformycin. This is probably the most active agent against hairy cell leukemia producing over 90% responses with minimal toxicity with good performance status patients. CTEP is organizing randomized trials of Deoxycoformycin against Interferon in both the splenectomized and nonsplenectomized settings.

2. Trimetrexate. There is preliminary evidence of activity of this drug in colon cancer. Randomized scheduling studies (daily X 5 versus single dose Q2 weekly) are being organized in both colon cancer (SWOG) and in head and neck cancer. A randomized comparison of Trimetrexate versus Methotrexate trial in head and neck cancer (ECOG) will be activated in September.

3. Tiazofurin. A study to explore the effect of dose/schedule on clinical efficacy and biochemical effects (e.g. TAD synthesis) was initiated in acute leukemia.

4. Acivicin (AT-125). Aminosyn, an amino acid preparation was shown to rescue CNS toxicity of Acivicin, allowing higher doses to be given in animal models. A Phase I study now beginning in man.

5. Mitoxantrone. The FDA Oncologic Advisory Committee in March recommended FDA approval for treatment of metastatic breast carcinoma. The original studies demonstrating its activity were conducted under NCI sponsorship at M.D. Anderson and Ohio State University.

6. Carboplatin. Comparative study plus cytoxan versus cis platin plus cytoxan in ovarian carcinoma is now underway in SWOG.

7. Chlorozotocin. Under exploration as treatment for islet cell carcinoma of the pancreas.

8. HMBA. Two Phase I trials using the 5-day continuous infusion schedule now completed with metabolic acidosis and mental status changes being the dose limiting toxicities. Two Phase I trials are currently evaluating the 10-day continuous infusion schedule, and the exploration of biological endpoints as markers of differentiation are an intrinsic part of these trials. A Phase II trial in preleukemia is planned.

9. Didemnin B. Two Phase I trials are near completion. Prolonged nausea and vomiting has been the dose limiting toxicity. Currently both trials are escalating doses further with the use of combined antiemetic therapy.

10. Pibenzimol. Two Phase I trials using the 5-day continuous infusion schedule are near completion; hypoglycemia second to pancreatic injury is the dose limiting toxicity. Disease specific Phase II trials in patients with pancreatic cancer are soon to the activated.

11. Merberone. Two Phase I trials using the 5-day continuous infusion schedule are in progress. Mild peripheral phlebitis has been the only toxicity observed to date.

12. Dihydrolenperone. One Phase I trial under progress. This is the first compound brought to clinical trial exclusively on activity against human cell lines.

13. AZQ. A new Phase I trial of AZQ using 24-hour continuous infusion schedule has been activated, based upon an improved therapeutic index seen in cellular pharmacology studies. This schedule may be used in a comparative randomized efficacy trial against BCNU as the frontline treatment of brain tumors.

14. Ifosfamide. Ifosfamide in combination with VP-16 and cis platin has demonstrated activity in refractory testicular carcinoma. The drug is extremely active as a single agent in sarcomas, and an intergroup sarcoma randomized trial is planned of Ifosfamide, Adriamycin, and DTIC versus Adriamycin and DTIC. Other trials are testing for a lack of cross-resistance with cyclophosphamide in sarcomas.

15. Menogaril. Phase II trials are ongoing in a large number of disease sites.

16. Taxol. Phase I evaluation of Taxol is almost complete. Anaphylactic reaction have been virtually eliminated by the use of a 24-hour continuous infusion in patients who have been premediated with steroids, cimetidine, and diphenhydramine hydrochloride. Dose limiting toxicities appear to be myelosuppression and peripheral neuropathy. Phase II trials began at the end of the year.

17. SR-2508. This 2-nitroimidazole analog of misoindazole recently completed Phase I evaluation. The dose limiting toxicity is peripheral neuropathy. SR-2508 can be given approximately seventeen times during a six week period in a dose which effects a similar sensitizer enhancement ratio as misonidazole, which can be given only six times during a course of similar length. This has encouraged the establishment of a large randomized trial in head and neck cancer, comparing radiotherapy with and without SR-2508.

DRUG MANAGEMENT AND AUTHORIZATION SECTION

Drug Accountability. The drug accountability system, implemented in January 1983 has continued to function well. All investigational drugs must be ordered and dispensed to patients by protocol and documented on a drug accountability form. Drug accountability has proven to be an essential addition to the site visit monitoring as conducted by the Quality Assurance and Compliance Section. The Drug Accountability Records Form and instructions were approved by the Office of Management and Budget as an official form. In addition, the form is being accepted by several drug companies. In the past year, the form and instructions

were professionally printed into a concise booklet which was sent to investigator and pharmacists.

Drug Distribution and Computer Modifications. The Drug Distribution and Protocol Monitoring System (DDPMS) has undergone several computer and support enhancements in the past year. These include:

1) The redesign of the investigator name and address file.

2) Protocol expiration dates were redefined to better match the needs of each study phase and drug distribution mechanism. Special Exceptions protocols that had not been used in six months have been inactivated and Group C protocols that have not been used in twelve months have been inactivated.

3) Cooperative Groups were asked to provide lists of their investigators so that DMAS could insure that all members have submitted Investigator Registration forms. This reduced the repetitive submission of FDA-1573 forms for investigators who may have already been registered with NCI under other drug distribution mechanisms minimizing form duplications and has save both NCI and Cooperative Group personnel and computer time.

4) The DDPMS aided in reducing starter supplies of drugs sent to investigators for new protocols by providing data showing the extent that starter supplies were dispensed to patients. Analysis of this data revealed that less drug could be provided initially without compromising patient treatment. This exercise contributed to drug distribution cost savings. The survey indicated that $100,000 in drug start up costs could be reduced by 80-90%.

Site Visits to Investigators. The drug accountability system has been site visited in several institutions, usually in conjunction with the medical site visit. The drug accountability system has provided a new dimension in the tracking of investigational drugs.

Special Exception (Compassionate) Use of IND Drugs. The administrative management of the Special Exception protocol drug distribution system is part of this Section. A written set of policies and procedures governing the use of Special Exception drugs are provided to prospective management compliance.

These guidelines have provided the necessary information for physicians to improve their compliance in returning the investigational protocol for submission with the FDA.

Investigator Annual Registrations. Administrative of the annual registration of investigators continues to be an important function of DMAS. The compliance is presently 100%. In the past year we have taken steps to limit the multiple submissions of FDA-1573 forms for the same investigator as indicated under Drug Distribution Modifications.

Drug Cost Containment. Cost containment has been achieved through the closer scrutiny of drug requests and by continuing to reduce the distribution of commercially available drugs. The procedures for ordering Erwinia Asparaginase one of the msot expensive drugs in the program, were changed so that drugs were requested for specific patients. This minimizes stocking of the drug and should result in a marked decrease in future distribution. In FY85, Group C, and and Special Exception drug distribution. In implemented. These improved procedures for handling Group C and Special Exception drugs have resulted in decreased drug cost, $51,216 and $84,039, respectively. These and other procedures have significantly reduced the total drug budget. The savings for FY'84 was $1,402,467 and for the 1st and 2nd quarter FY'85 $224,548 as compared to the immediate prior period of time.

The following are some of the drug distribution data for the past year.

Total Number of Drugs Orders	Total Number of Line Items per Drug Request	Total Number on Group C Drugs Request	Total Number for Special Exceptions
11,361	18,124	1,665	2,148

Special attention is given to making THC, a Schedule I controlled substance, available for patient treatment. In 1985, the following statistics apply to the THC antiemetic program:

THC-CY'85

No. of Hospital Pharmacies Participating		601
No. of Physicians Registered to Prescribe THC		2,538
No. of Capsules Distributed	2.5 mg.	40,600
	5.0 mg.	153,000
Approximate No. of Patients Receiving THC	2.5 mg.	964*
	5.0 mg.	3,747*
No. of State Sponsored Pharmacies Receiving THC		65
No. Individual Investigators Receiving THC		2

* If patients received THC in more than one calendar-quarter, they are counted more than once. If patients received 2.5 and 5.0 mg. capsules, they are counted more than once.

Management techniques have been applied to improve the inventory control for THC, thus reducing pharmacy investigators as well as reducing overall THC costs. This drug has been rescheduled and will be marketed commercially in the summer of 1986. A smooth transition of distribution to the commercial supplier is anticipated this summer.

STAFF PUBLICATIONS

1. Chun, H., Davies, B., Hoth, D., Suffness, M., Plowman, J., Flora, K., Grieshaber, C., and Leyland-Jones, B: Didemnin B: The First Marine Compound Entering Clinical Trials as an Antineoplastic Agent. Invest. New Drug, 4: 1986. In press.

2. Chun, H., Hoth, D., and Leyland-Jones, B: Spirogermanium Hydrochloride: Current Status and Prospects. Cancer Treat. Rpts. In press.

3. Chun, H., Leyland-Jones, B., Caryk, S., and Hoth, D: Central Nervous System Toxicity of Fludarabine Phosphate. Cancer Treat. Rpts. In press.

4. Chun, H., Leyland-Jones, B., Hoth, D., Shoemaker, D., Wolpert-DeFilippes, M., Greishaber, C., Davignon, J.P., Moon R., Rifkind R., and Wittes, R.: Hexamethylene Bisacetamide: A Polar-Planar Compound Entering Clinical Trials as a Differentiating Agent. Cancer Treat. Rpts. In press.

5. Estey, E., Hoth, D., Simon, R., Marsoni, S., Leyland-Jones, B., and Wittes, R: Therapeutic Response in Phase I Trials of Antineoplastic Agents. Cancer Treat. Rpts. In press.

6. Foster, B., Clagett-Carr, K., Hoth, D., and Leyland-Jones, B: Gallium Nitrate: The Second Metal with Clinical Activity. Cancer Treat. Rpts. In press.

7. Foster, B., Clagett-Carr, K., Hoth, D, and Leyland-Jones, B.: Pentamethylmelamine: Review of an Aqueous Analog of Hexamethylmelamine. Cancer Treat. Rpts., 70: 383-389, 1986.

8. Foster, B., Clagett-Carr, K., Leyland-Jones, B., and Hoth, D.: Results of NCI-Sponsored Phase I Trials with Carboplatin. Cancer Treat. Revs., 12: 43-49, 1985.

9. Foster, B., Clagett-Carr, K., O'Dwyer, P., and Leyland-Jones, B.: Letter to the Editor Hypoglycemia: Not An Important Side Effect of MGBG. Cancer Treat. Rpts., 64: 1340, 1985.

10. Foster, B., Clagett-Carr, K., Shoemaker, D., Suffness, M., Plowman, J., Trissel, L., Greishaber, C., and Leyland-Jones, B.: Echinomycin: The First Bifunctional Intercalating Agent in Clinical Trials. Invest. New Drugs, 3: 403-410, 1986.

11. Leyland-Jones, B. and O'Dwyer, P: Biochemical Modulation - Application of Laboratory Models in the Clinical. Cancer Treat. Rpts., 70: 219-229, 1986.

12. Mauer, J., Hoth, D., Macfarlane D., Hammershaimb, L., and Wittes, R.: Site Visit Monitoring Program of the Cooperative Groups: Results of the First Three Years. Cancer Treat. Rpts., 69: 1177-1187, 1985.

13. Wittes, R., Marsoni, S., Simon R., and Leyland-Jones, B: Editorial, The Phase I Trial. Cancer Treat. Rpts., 69: 1235-1239, 1985.

REGULATORY AFFAIRS BRANCH

The Regulatory Affairs Branch is responsible for preparing and submitting investigational new drug (IND) applications to the Food and Drug Administration (FDA) for initiating clinical trials and to meet all FDA regulatory requirements pertaining to these agents. In addition the Regulatory Affairs Branch implements, coordinates and administers the monitoring of clinical trials with anticancer agents sponsored by the Division of Cancer Treatment, NCI. The Branch assures that clinical trials are conducted according to NIH and NCI policies and procedures and Federal regulations.

The Regulatory Affairs Branch is composed of the Drug Regulatory Affairs Section and the Quality Assurance and Compliance Section. The Drug Regulatory Affairs Section is responsible for:

1. Liaison between the Division of Cancer Treatment, NCI, and both the Office of Drug Research and Review and Office of Biologics Research and Review of the FDA;

2. Submission of INDs to FDA after analyzing the adequacy of the data for cytotoxic and biological agents;

3. Coordination of responses to correspondence from FDA regarding IND applications and amendments;

4. Compliance with adverse drug reaction regulations;

5. Liaison with the preclinical sections of the Division of Cancer Treatment, particularly the Developmental Therapeutics Program and the Biological Response Modifiers Program;

6. Liaison with pharmaceutical companies to provide preclinical and clinical data and any other information required to complete approval for New Drug Applications; and

7. Liaison with intramural clinical groups in NCI and NIH on regulatory issues concerning agents of particular interest.

The Quality Assurance and Compliance Section is responsible for:

1. Planning, organization and administration of a program for monitoring the quality assurance of clinical data for all clinical trials with anticancer agents sponsored by the Division of Cancer Treatment;

2. Attendance at 10-20% of site visits performed by the Cooperative Groups;

3. Carrying out the site visit audits of cancer centers and other single institutions conducting clinical research utilizing DCT-sponsored investigational agents;

4. Serving as the Project Officer for a contract with the Clinical Trials Monitoring Service;

5. Serving as an educational resource to the cancer community for site visit monitoring and regulatory requirements for clinical trials; and

6. Review of informed consent documents.

The professional staff of the Regulatory Affairs Branch includes the following individuals:

Dale Shoemaker, Ph.D., Chief

Drug Regulatory Affairs Section -
Dale Shoemaker, Ph.D., Acting Head
Howard Holden, Ph.D.

Quality Assurance and Compliance Section -
Dorothy Macfarlane, M.D., Head
Joan Mauer, B.S., M.T.
Jane Cassidy, R.N., B.S.

A summary of the activities for FY'86 includes:

1. Fourteen INDs for 17 cytotoxic and biologic agents were prepared and submitted to the Office of Drug Research and Review and Office of Biologics Research and Review of the FDA.

2. The INDs for four agents were discontinued due to a lack of clinical activity.

3. The data from all adverse drug reactions reported to the FDA since August 1, 1985 have been entered into a data base on a personal computer. Reports were prepared and distributed to the Drug Monitors and other staff in CTEP to help in their evaluation of agent toxicities. During CY'85 259 adverse drug reactions were reported to FDA.

4. The Quality Assurance and Compliance Section prepared, published and presented a paper on the results of site visit monitoring in the Cooperative Groups from 1982 through 1984.

5. Site visits were made to eleven cancer centers or other single institutions which are conducting trials with DCT sponsored investigational agents.

6. Meetings were held with the Division of Anti-Infective Drug Products of FDA to determine the preclinical data and the IND format required for the agents used to treat patients with AIDS.

7. Procedures were established for the monitoring of multi-institutional pediatric Phase I studies carried out by the pediatric Cooperative Groups (CCSG, POG).

DRUG REGULATORY AFFAIRS SECTION

IND Submissions.

For the FY'86, a Notice of Claimed Investigational Exemption for a New Drug (IND) was submitted to the Office of Drug Research and Review, Food and Drug Administration (FDA), for each of the following five compounds:

Drug	NSC Number
Photofrin II™	NSC 603062
Fazarabine (Ara AC)	NSC 281272
Oxanthrazole	NSC 349174
4-Ipomeanol	NSC 349438
Chloroquinoxaline Sulphonamide	NSC 339004

INDs were submitted to the Office of Biologics Research and Review, FDA, for the following biological response modifier agents:

Drug	NSC Number
Murine Monoclonal Antibody to Bombesin	NSC 377527
Human Monoclonal Antibodies to Human Colorectal Cancer	NSC 603569 NSC 605542
Murine Monoclonal Antibody to Reed-Sternberg Cells	NSC 603573
Murine Monoclonal Antibodies to T Lymphocytes for In Vitro Treatment of Bone Marrow	NSC 603570 NSC 603571 NSC 603572

Cross-filed INDs were submitted to FDA for the following agents:

Drug	NSC Number
Monocloncal Antibody to GD3 Ganglioside	NSC 608918
Retinoic Acid	NSC 329481
Trimetrexate	NSC 352122
Tumor Necrosis Factor (Genentech)	NSC 604175
Zoladex	NSC 606864

INDs Discontinued.

INDs for the following agents were discontinued due to a lack of clinical activity:

Drug	NSC Number
Desmethylmisonidazole	NSC 261036

Interferon: Immune (Meloy)	NSC 354655
Interferon: Leukocyte (Meloy)	NSC 335044
Streptonigrin	NSC 45383

The Regulatory Affairs Branch currently maintains 123 active INDs for both cytotoxic and biologic compounds.

Adverse Drug Reaction Reporting.

The Branch is responsible for reporting adverse drug reactions to FDA. During CY'85 259 adverse drug reactions were reported to FDA. A standard adverse drug reaction report form has been established and implemented. The data from these forms are being entered into a data base on a personal computer.

QUALITY ASSURANCE AND COMPLIANCE SECTION

The Quality Assurance and Compliance Section is responsible for on site monitoring of all clinical trials sponsored by the Cancer Therapy Evaluation Program. This includes all trials conducted by the Cooperative Groups, and studies conducted at cancer centers or other individual institutions which utilize DCT-sponsored investigational agents.

Cooperative Group Site Visits.

In the case of the Cooperative Groups, NCI has delegated the responsibility for organizing and conducting the monitoring program to each group. Each institution is to be monitored at least once every three years. During the past year, the Cooperative Groups site visited 118 member institutions, 141 affiliates and 10 CCOPs.

The Quality Assurance and Compliance Section continues to co-site visit with the Cooperative Groups in 10-20% of the scheduled visits to assure the adequacy of the audit procedures. In addition, the Cooperative Groups submit a report on each monitoring site visit to the Section for review. In October 1985, the Section published a paper summarizing the results of the first three years of Cooperative Groups monitoring. These data were also presented to the DCT Board of Scientific Counselors and the FDA Division of Scientific Investigations.

Phase I and Single Institution Site Visits.

The Quality Assurance and Compliance Section directly oversees the monitoring of Phase I and cancer center studies. Phase I studies are monitored three times per year. During the past year, 11 cancer centers or other single institutions which are conducting trials with DCT-sponsored investigational agents were site visited. Procedures were developed for the monitoring of multi-institutional pediatric Phase I trials to be conducted by the two pediatric Cooperative Groups.

Publications.

Mauer, J.K., Hoth, D.F., Macfarlane, D.K., Hammershaimb, L.D. and Wittes, R.E.: Site Visit Monitoring Program of the Clinical Cooperative Groups: Results of the First 3 Years. Cancer Treat. Rep. 69: 1177-1187, 1985.

SUMMARY REPORT
ASSOCIATE DIRECTOR FOR THE RADIATION RESEARCH PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE
OCTOBER 1, 1985 - SEPTEMBER 30, 1986

I. INTRODUCTION

In 1982 the Radiation Research Program (RRP) was established in the Division of Cancer Treatment (DCT), National Cancer Institute (NCI), National Institutes of Health (NIH). It has two Branches: the Diagnostic Imaging Research Branch (DIRB) and the Radiotherapy Development Branch (RDB). The mission of the Radiation Research Program is the planning, development, administration, and evaluation of an extramural radiation research program by means of establishing program priorities, allocating resources, maintaining project integration, evaluating program effectiveness, and representing the program in the management and scientific decision making processes of the National Cancer Institute. This requires the coordination of research activities with related programs at NCI and NIH, with other Federal agencies, and with national and international research organizations. A major goal of the DIRB is to maintain effective working relationships with the other divisions of NCI and with other NIH institutes which fund research in diagnostic imaging. The RRP also provides a focal point for radiation research for extramural investigators both nationally and internationally.

II. PERSONNEL

A. Staffing

1. Office of the Associate Director

John E. Antoine, M.D., Acting Associate Director
Ethel L. Meyer, Secretary to Associate Director
Jan Johnson, Technical Assistant
Theresa Phillips, Clerk-Typist

2. Administrative Office

Barbara Vermillion, Administrative Officer
Barbara Levin, Budget Assistant

3. Diagnostic Imaging Research Branch

Francis Ruzicka, M.D., Chief
Matti Al-Aish, Ph.D., Deputy Chief
Roger Powell, Program Director
Ann Malner, Branch Secretary

4. Radiotherapy Development Branch

Francis Mahoney, Ph.D., Acting Chief
Robert Morton, M.S., Program Director
Thomas Strike, Ph.D., Program Director
Sandra Zink, Ph.D., Cancer Expert
Maureen Volz, Statistical Assistant

B. Recruitments

Chief, Radiotherapy Development Branch
Vacant, Radiation Oncologist, Radiotherapy Development Branch
Vacant, Secretary, Radiotherapy Development Branch

III. MAJOR ACTIVITIES

The Radiation Research Program has continued to develop research in areas of imaging and treatment. At the February 1986 Division of Cancer Treatment Board of Scientific Counselors meeting, presentations were made highlighting advances in diagnostic imaging. Recent developments in magnetic resonance imaging and spectroscopy, positron emission tomography, single photon emission computerized tomography, and computerized tomography were discussed. Also at the Board of Scientific Counselors meeting a Program Announcement was presented for specific cell diagnosis by imaging employing radiolabeled substances. An RRP sponsored workshop on that topic was held in Bethesda, Maryland, April 3-4, 1986. In May of 1986, an RFA entitled, "National Collaborative Diagnostic Imaging Trial Projects" was published. It is intended for FY 1987 funding. The object of the RFA is to support multicentered cooperative clinical trials to determine the most effective imaging procedures needed to diagnose, stage and monitor carcinoma of the prostate and lung.

The neutron therapy clinical trials showed continued improvement and a formal status report of this research effort was given to the May 1986, Division of Cancer Treatment Board of Scientific Counselors meeting. Equipment problems have been overcome and patient accrual is increasing. Phase III head and neck and prostate carcinoma protocols continue to accrue in patients. Protocols to study cervix carcinoma and non-oat cell carcinoma of the lung are presently proceeding through the review process. Also, at the May 1986 DCT Board of Scientific Counselors meeting, concept approval was given to a Patterns of Care study with emphasis to be in the areas of breast carcinoma and the gynecological tumors. Three contracts to evaluate electron beam treatment planning were awarded. The application of computers in the delivery of radiotherapy was addressed in a workshop. In order to improve the development of deep heating in the field of hyperthermia, a workshop on this topic was sponsored by the RDB.

IV. RADIATION RESEARCH PROGRAM RESEARCH GRANT AND CONTRACT SUPPORT

FY86 BUDGET
(Dollars in Thousands)

	Contracts Number/Amount	Grants Number/Amount
Diagnostic Imaging Research Branch	6/1,358	142/24,347
Radiotherapy Development Branch	23/3,140	211/41,712
TOTAL RRP	29/4,498	353/66,059

V. SCIENTIFIC OVERVIEW

DIAGNOSTIC IMAGING RESEARCH BRANCH

The DIRB has continued to show satisfactory growth. Starting as a Branch in 1982 with 31 funded grants, this number had increased to 110 in 1985 and has reached 120 traditional (R01) grants this year. There are six continuing Program Projects (P01). The sixth Program Project (not included as yet in the budget) initiated this year deals with the timely topic of monoclonal antibodies for imaging. There are also a total of five Conference and New Investigator Grants, and 12 Small Business Innovative Research (SBIR) grants (See Table below). In the category of specifically directed Program support, there are six ongoing contracts (nuclear magnetic resonance) and four RFA (included under P01s in the Table) initiated grants (T1, T2 Relaxation Study) that complete the budget.

The following is the DIRB estimated budget for FY86:

FY86 DIRB BUDGET

GRANTS		$ (Mil)
Traditional (R01)	120	19,153
Program Projects (P01)	5	3,519
Conf. and New Investigator (R13 & R23)	5	230
SBIR	12	1,445
TOTAL GRANTS (estimate)	142	$24,347
CONTRACTS		
NMR	6	1,358
SBIR*	--	--
TOTAL CONTRACTS (estimate)	6	1,358
Total DIRB Budget (FY86) (estimate)	148	$25,705

*FY86 SBIR contract awards not yet determined. Late in FY85 14 SBIR contracts were funded for $82,000.

In FY 85, with a total number of 110 grants, the total support figure was $18,616,000 (actual). In 1986, there has been an increase of 32 grants with a total of 142. The total figure has risen to $25,705,000 (estimated), an increase of approximately $7 million.

GRANTS

Nuclear Medicine

Of the 120 traditional grants and 6 Program Projects in Diagnostic Imaging and Nuclear Medicine, Nuclear Medicine investigation has the largest number (43 grants) involving research with radiopharmaceuticals, monoclonal antibodies, physics/equipment development and biological studies. The major efforts in new radiopharmaceuticals center about positron emission tomography (PET), single photon emission computed tomography (SPECT), and monoclonal antibodies. PET studies are increasingly and successfully being utilized to determine the regional metabolism of various diseases.

Conventional X-Ray

Conventional x-ray technology is next in number (36 grants) where concentration is mainly centered on physics/equipment and technological development, especially digital radiography. Contrast agents, perception, cognition and display of images continue to be areas of investigational activity.

Magnetic Resonance

There are 27 grants that support research in magnetic resonance imaging and/or spectroscopy. These are concerned with physics/equipment development, paramagnetic contrast agents and various biological and clinical studies. With respect to clinical/biological applications, an example is the differentiation of sodium in extracellular versus intracellular locations relating to tumors and ischemia. This technology is making possible the in vivo grading of brain tumors.

The employment of higher strength magnetic fields (1.5 Tesla or above) has made possible the imaging of nuclei other than hydrogen (e.g., phosphorus (^{31}P), fluorine (^{19}F) and sodium (^{23}Na). This technology is being further developed to differentiate tissues for diagnostic purposes.

The use of paramagnetic contrast substances, again for increasing specificity of diagnosis, is being studied. Substantial progress is being made in this research area.

Ultrasound

Twenty grants support research in ultrasound. Again, the major goal is directed toward tissue diffentiation. A significant effort is being made in developing advanced ultrasound imaging systems, e.g., computed ultrasound tomography for the detection of breast disease.

DIAGNOSTIC IMAGING RESEARCH BRANCH

RRP SUPPORTED RESEARCH

1) Comparative Clinical MRI Studies (RFP NCI-CM-37564-27)

   Five Institutions are participating in this contract (now in its last year of funding). Case accrual is satisfactory. Double blind review of case material is being carried out at a central location and computer entry of data has begun. Seven protocols are operative: Brain Neoplasm, Lung Cancer, Uterine Neoplasm, Liver Metastases, Musculo-skeletal Tumors, Congenital Heart Disease and Cervical Myelopathy.

2) Basic Research in Factors Influencing Nuclear Magnetic Resonance Relaxation Times in Biological Tissues. (RFA-84-CA-16)

   Four awards were made, and research has begun during the past year. This magnetic resonance study is an investigation of the basic principles underlying the relaxation times of various body tissues by employing varying field strength and radiofrequency pulsing. The goal is differentiation of normal from abnormal tissues.

3) A mechanism (Cooperative Agreement), new for support of clinical trials in diagnostic imaging research, has been announced and an RFA has been published (RFA-86-CA-10). The RFA is entitled "National Collaborative Diagnostic Imaging Trial Projects." This introduces the concept of the Radiology Diagnostic Oncology Group (RDOG). This will be funded in FY87. The objective of the RFA is to support multicenter cooperative clinical trials to determine the most effective imaging procedures needed to stage and monitor carcinoma of the prostate and lung. The result of this project would be the development of specific algorithms for the appropriate sequential use of state-of-the-art imaging procedures in the diagnosis, staging, and follow-up of these specific neoplasms.

SMALL BUSINESS INNOVATIVE RESEARCH GRANTS AND CONTRACTS

This funding area has been increasingly active with many excellent applications and proposals suitable for support by Radiation Research Program. Indeed, it has has received the largest number of applications in the National Cancer Institute.

In the Diagnostic Imaging Research Branch there are 14 funded grants. Contracts for FY86 have not yet been awarded. Investigations range from instrument development leading to image improvement in all modalities to the development of radiopharmaceuticals and contrast agents. Details are found in the Diagnostic Imaging Research Branch report.

RADIOTHERAPY DEVELOPMENT BRANCH

The Radiotherapy Development Branch continues to administer a large program of basic science and clinical research related to cancer treatment. The disciplines represented are radiation oncology, radiobiology, radiation chemistry and radiation physics.

Major areas of funding are in particle radiation therapy, hyperthermia and associated biology, radiosensitizers, intraoperative radiation therapy, photodynamic therapy and radiation physics.

Particle Radiotherapy

Support of particle radiation therapy constitutes the largest portion of the RRP budget. Included are investigations at four neutron facilities, one proton facility, and one helium/heavy ion facility. Neutron researchers are continuing Phase III clinical investigations. Data strongly support neutron therapy as the treatment of choice in salivary gland tumors and favorable results are also being reported in carcinoma of the prostate. Research with heavy ions, helium ions, and protons is progressing as planned. Proton beam therapy appears to be extremely effective in treating eye melanoma, preclival chordomas and chondrosarcomas of the base of the skull.

Hyperthermia and Radiotherapy

Encouraging results are being reported in the utilization of regional hyperthermia used in conjunction with radiation. Basic and clinical research continues to receive significant support from RRP. Phase I and II clinical research shows the combined use of radiation therapy and hyperthermia to be effective in the control of advanced local/regional disease. Limited Phase III trials in superficial tumor sites are being developed. Deep heating continues to be a problem and equipment improvements will be necessary. Research in noninvasive thermometry is continuing; however, at this point in time thermometry requires invasive probes.

Intraoperative Radiation Therapy

Intraoperative radiation therapy continues to show promise in the treatment of deep seated malignancies. The combination of surgery in conjunction with intraoperative radiation has been proven to be technically possible with acceptable morbidity in lesions where normal tissue tolerance usually limits the total dose tumor. Some disease sites presently being studied are pancreatic carcinoma, retroperitoneal sarcomas, gastrointestinal neoplasms, and areas of lymph node metastases. Other regions are being considered for treatment by this research modality.

SCIENTIFIC OVERVIEW - (Continued)

RADIOTHERAPY DEVELOPMENT BRANCH

Radiosensitizers

Basic research with several radiosensitizers is ongoing. The screening program has produced two non-nitro classes of compounds: the quinoxyline line and the benzamide line. SR-2508, a nitro-imidazole in clinical trials, has been found to be less toxic (peripheral neuropathy) than misonidazole.

Clinical trials to evaluate misonidazole as a chemosensitizer in combination with chemotherapeutic agents are being developed.

Photodynamic Therapy (PDT)

Research in this field is active. A di-hematoporphyrin ester with an affinity for plasma lipoproteins has been identified as an active tumor localizing component of hematoporphyrin. This will permit pursuit of more effective analogues which have less skin photosensitization than currently used hematoporphyrins. Skin photosensitization has been the major toxicity associated with PDT.

The biochemical mechanisms involved in tumor cell cytotoxicity are also being investigated. Mitochondrial function can be severely injured by PDT in a dose and time related relationship. It would appear that the hydrophobic environment of the mitochrondrial membrane is conducive to localization of hematoporphyrin and that impaired mitochondrial function may offer the basis for the cytotoxicity that occurs.

Hyperthermia has been found to be synergistic with PDT, especially when given immediately post-PDT.

Phase I and II clinical trials have shown local control and palliation in certain endobronchial, head, neck, bladder and eye tumors. Several institutions feel that limited site-specific and tumor-specific Phase III randomized clinical trials should be performed.

Radiation Physics

Continuing support is provided for the characterization of external photon and electron beams. Data continues to indicate that there are different microdosimetric properties in the deposition of the energy from these beams in tissue. The dosimetry of particle beams is being further developed. Computer utilization in the delivery of radiation beams and in radiation treatment planning continues to increase.

The following is the RDB estimated budget for FY86:

FY86 RDB BUDGET

GRANTS		$ (Mil)
Traditional (R01)	185	29,532
Program Projects (P01)	9	11,235
Conference and New Investigator (R13 & 23)	11	535
SBIR	6	410
TOTAL GRANT (estimate)	211	41,712
CONTRACTS		
Regular	23	3,140
SBIR*	--	--
	23	3,140
TOTAL RDB Budget (FY86)	234	$44,852

*FY86 SBIR contract awards not yet determined. Late in FY85 14 SBIR contracts were funded for $916.

WORKSHOPS:

DIAGNOSTIC IMAGING RESEARCH BRANCH

One Workshop and one Mini-Workshop were conducted during FY86. The Workshop was entitled "Cell Specific Diagnosis Employing Radiolabeled Agents" and was held on April 3-4, 1986 in Bethesda, Maryland. This meeting dealt with both radiolabeled monoclonal antibodies and radiolabeled specific binding substances as they relate to imaging. These are promising agents for detection, staging and treatment of cancer. Recommendations for further research are included in the section of the DIRB Report.

The Mini-Workshop, directed toward the same goal of sensitivity and specificity of diagnosis was held in Hartford, Connecticut, on May 5, 1986. It was entitled "Tissue Characterization by Radiologic Imaging: Regional Metabolism and Tissue Perfusion." This meeting was held in preparation for a full scale workshop on the same subject to be held on October 9-10, 1986 (FY87). The agenda of this meeting will be designed for the determination of function in specific anatomical sites, employing the more recent advances in technology such as magnetic resonance imaging and spectroscopy, positron emission tomography and digital radiography.

WORKSHOPS:

RADIOTHERAPY DEVELOPMENT BRANCH

During FY 1986, the RDB sponsored two workshops on topics of potential future solicited research. These were: (1) "New Directions in Computer-Assisted Radiotherapy," and (2) "Localized Heating of Deep Seated Tumors."

"New Directions in Computer-Assisted Radiotherapy"

Advances in the computer field in recent years have brought about a revolution in the solution of problems that can be accomplished with computers and brought forth new researchers interested in application of computers in medicine. Artificial intelligence is currently being used in scientific medicine to develop and create expert systems. These highly specialized computer systems are being developed to provide the medical profession with a broad knowledge base, which is then utilized through appropriate decision trees to arrive at a specific end point. Systems are currently in use for the testing of pulmonary function, chemotherapy protocol management, and in the diagnosis of disease. An NCI-sponsored workshop was held in September 1986, to assess the applicability of these advanced computer techniques to the problems faced in Radiation Oncology.

"Localized Heating of Deep Seated Tumor"

Currently the development of the field of regional hyperthermia is hindered because of the difficulty in deep heating with the presently available deep heating devices. Clinical results of superficial tumors treated with hyperthermia in conjunction with radiotherapy are very encouraging but evaluation of the combination of modalities awaits development of improved deep heating units. Presently there is a serious question whether local deep heating is technically and physiologically possible. This workshop focused on this problem and assisted in finding answers to the problem.

VI. FUTURE RADIATION RESEARCH PROGRAM DIRECTIONS

Diagnostic Imaging Research Branch: This Branch will continue to stimulate research to improve the sensitivity and specificity of diagnosis by imaging and related methodologies, (e.g., magnetic resonance imaging, and spectroscopy, SPECT, radiolabeled monoclonal antibody techniques, radiolabeled cell receptor techniques). A unifying theme of DIRB research is to pursue tissue specific diagnosis in a noninvasive manner.

There will be promotion of multi-institutional imaging trials. The cooperative group method of investigation permitting the participation of multiple institutions in specific protocols will facilitate a more rapid accumulation of data and evaluation of imaging information with a subsequent improvement of diagnostic sensitivity and specificity.

Radiotherapy Development Branch: The particle radiation therapy program will continue to be a high priority research area for the near future. The promise of improved therapeutic results in specific disease sites will continue to be explored.

The use of radiation and hyperthermia continues to be encouraging but further research needs to be performed in the development of deep heating units and of noninvasive thermometry.

Radiosensitizers have demonstrated an ability to increase the sensitivity of neoplastic tissue to radiation beams and attempts will be made to improve the efficacy of these agents and to decrease their toxicity.

Photodynamic therapy is less well developed than the traditional disciplines of radiation oncology but because of promise in this area, further research efforts will be stimulated.

Intraoperative radiation therapy research has progressed to a point where Phase III trials in specific disease sites need to be performed.

The dosimetry of nuclide tagged monoclonal antibodies in an imporant research area of this rapidly developing therapeutic approach and requires further support.

Other radiation research initiatives will be developed independently and by utilization of the Research Plan for Radiation Oncology - 1987.

FY86 Annual Report Summary
Diagnostic Imaging Research Branch

The Diagnostic Imaging Research Branch (DIRB), Radiation Research Program (RRP), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), supports and administrates research leading to the development of radiologic instrumentation and methodology to improve diagnosis of cancer and other diseases. The ultimate objective is non-invasive cell specific, anatomical and functional diagnosis of cancer and other diseases.

Several modalities both ionizing and non-ionizing are employed in the field of diagnostic imaging which include diagnostic radiology and nuclear medicine. In the "Ionizing" Section, equipment development and especially digital radiography are receiving much attention resulting in improved x-ray technology. In the non-ionizing group (magnetic resonance imaging/ spectroscopy and ultrasound) much activity is supported.

In Nuclear Medicine new radiopharmaceuticals and the technologies of positron emission tomography (PET), single photon emission computed tomography (SPECT) and radiolabeled monoclonal antibodies and specific binding substances are being investigated.

A variety of contrast agents for imaging which include the new less-toxic contrast media, paramagnetic agents for magnetic resonance imaging and contrast agents for use with ultrasonography are being researched.

GRANTS

The grant awards for fiscal year 1986 number 144. The latter includes 120 traditional R01 Grants, 5 Program Projects, 5 Conference and New Investigator Grants and 14 Small Business Innovative Research Grants. This represents an increase of 32 Grants over the previous year. The total cost estimated for FY86 is $25,680,000 as opposed to the 1985 cost of $18,616,000 (actual) which supported 110 grants.

Of the 126 traditional R01 Grants and Program Projects, 36 grants support conventional x-ray technology including computed tomography, and 27 grants support research in magnetic resonance imaging and spectroscopy. Forty-three grants support work in nuclear medicine and 20 grants underwrite ultrasound research.

Ionizing Technology

In the field of x-ray diagnosis, there is major support for work on improvement in technology and development of equipment. There is emphasis on digital radiography. Work continues on various contrast agents and there are 9 grants supporting studies of perception, cognition and display of images. Practically all grants deal with the biological/ clinical sphere.

Nuclear medicine has a total of 43 grants, 12 being in the development of radiopharmaceuticals, 12 in the field of imaging employing monoclonal antibodies and receptor specific binding substances, 10 with the development of physics/equipment and 9 grants are primarily engaged with biological and clinical problems.

X-Ray Diagnosis

Achieving high quality radiographs for cancer diagnosis in the chest is a major goal of DIRB. By using a nonuniform field of radiation to compensate for variation in tissue thickness over the chest, a grantee in our program was able to show that this approach resulted in (1) superior images, (2) higher true negative readings and (3) lower false positive readings. A computer simulation of this system has shown that films with higher gradient peak could significantly improve the contrast of soft tissue structures in the chest. The digitization process is used in this study.

In conventional chest radiography, the wide range of x-ray transmission through the chest makes it impossible for the film to optimally display lesions in all regions of the chest. Another grantee has developed a computerized heavy metal printer technique which, based on a low-dose electronic test image, facilitates a patient-specific x-ray beam filter. The use of the filter results in optimal image contrast in all regions as determined by experiments involving test objects. Such improvements in chest x-ray will permit earlier detection of lung cancer, leading to improved diagnosis and prognosis of cancer.

Digital Imaging

Excellent progress has been made in the development of a high resolution, six-terminal console for the interactive retrieval and display of digitized images from radiography (and also MRI and ultrasound). Interest in digital radiography is rapidly increasing each year because of the added advantages which are possible once the image has been converted to photoelectronic form.

Nuclear Medicine

More than 35% of the total funded grants by DIRB relate to Nuclear Medicine. This represents the largest number of grants among our major categories.

One grantee is using enzyme inhibitors as radiopharmaceutical agents. The ultimate goal is to understand the potential and limitation of using radiolabeled enzyme inhibitors as imaging agents. The general application of this approach to specific disease such as cancer, where enzymatic activities are specifically increased or decreased, may be valuable for clinical diagnosis.

Research in the development of new radiopharmaceuticals goes beyond the imaging agents. One therapeutic agent (186-Re), a beta emitting radionuclide bonded to a disphosphate ligand which preferentially accumulates at the site of metastatic bone cancer, was found to have the potential for reducing the debilitating pain associated with bone cancer. Two dogs with naturally occurring bone cancer were treated with this agent and subsequently showed marked improvement in their condition. Further research to evaluate this agent and others with similar effects are in progress.

A new and important myocardial perfusion agent (Tc-99m-TBI) has been successfully tested in the human by one of our researchers. According to the investigator, this is the first time that a coordination complex labeled with this radionuclide (Tc-99m) has shown this effect in man. The research is now being directed toward purification, evaluation of the pharmacokinetic properties of the agent, and further studies of closely related members of this versatile class of complexes.

One grantee uses iodinated thiouracil (TU) or S-35-TU in therapy and diagnosis of malignant melanoma. Studies in murine melanoma have demonstrated in vivo distribution in primary and metastatic sites appropriate for successful diagnosis using iodinated TU. Further therapeutic application of S-35-TU in the mouse model has been successful in producing complete tumor remission. These techniques are expected to contribute significantly to the diagnosis and therapy of human melanoma.

The non-invasive measurement of arterial concentrations of PET tracers employing a pair of BaF^2 scintillators with sufficient time resolution to depict the contents of the cardiac chambers is another interesting example of PET research. The identification of this pre-arterial pool is assisted by multigating the time-of-flight (TOF) spectra, allowing the pulsatile cardiac motion to serve as a fingerprint of the intra-ventricular activity. This non-invasive alternative to arterial puncture should broaden the applicability of quantitative positron tomography.

Research in the use of monoclonal antibodies for cancer detection is increasing. Currently, our program supports several research grants in monoclonal antibody research. The following report was related to us by one of our grantees:

> "An exciting result in patient studies was the detection of an occult focus of choriocarcinoma in the chest of a 29 year old woman with a history of choriocarcinoma, a rising serum human chorionic gonadotropin (HCG), but no obvious source of disease. Multiple monoclonal scans were performed using an I^{131} label. This labeled antibody was intravenously administered. On days 2-5, a focus of increased radioantibody activity was seen on scans of the left chest. This was in a region of scarring which has been relatively stable in the left chest on multiple CT scans".

Such a finding indicates the importance of the use of monoclonal antibodies in cancer diagnosis in comparison to other modalities.

Non-Ionizing Technology

Magnetic Resonance

In the category of magnetic resonance imaging and spectroscopy, there are a total of 27 grants, 18 of which are involved with various biological studies, including tissue relaxation times, chemical shift imaging, and multinuclear imaging. Six grants are concerned with the development of

various paramagnetic contrast agents for use with magnetic resonance imaging and 3 grants are directly concerned with physics/equipment development.

New designs of radiofrequency coils to improve the image quality and speed of examination have been developed for MR imaging systems, and pulse sequences are being studied to improve the ability of MRI to characterize tissues following therapy. Several programs in MRS (Magnetic Resonance Spectroscopy), evaluating regional metabolism are specifically directed at the assessment of chemotherapy and radiation therapy. Organ-specific magnetic resonance contrast agents are under development and evaluation, and different investigators are looking at the toxicity of chelated paramagnetic ion contrast agents for MRI.

Others are investigating the uses of MRI in applications for the detection and prognosis of metastatic disease. Broad theoretical and applied studies are attempting to develop new MRI systems for faster imaging, three-dimensional imaging, and display of magnetic resonance data. Phantoms have also been developed by several investigators to provide means for quality control calibration of the operations of MRI systems from day to day.

Several projects which employ higher magnetic fields (1.5 Tesla or above) are capable of studying physiological and pathological properties of tissues both in vitro and in vivo, using different nuclear magnetic species such as phosphorus (^{31}P), fluorine (^{19}F), sodium (^{23}Na) and others. One program project has made particular progress in quantitatively assessing the amount of sodium present in the intracellular versus the extracellular spaces in the presence of tumors and ischemic tissues and visualizing them by sodium chemical shift imaging.

Ultrasound

Ultrasound has 20 grants in its program. Ten are involved with biological/clinical applications including tissue differentiation, 9 are applied to physics and equipment development and one is engaged with the study of contrast agents in ultrasound.

New improvements continue to be made in advanced ultrasound imaging systems, and their ability to detect tumors. Investigators are studying the propagation and scattering of acoustic energy in tissues and have reduced ultrasound "speckle" and increased spatial and contrast resolution in images by electronic signal processing and "front end" transducer and array designs. High speed ultrasonic imaging and reflex transmission ultrasound imaging systems are under development and evaluation.

Several projects have been developing protocol systems for ultrasound computed tomography of breast tissues. One of these projects has begun to use a Cray supercomputer to process millions of detailed corrections to ultrasound images because of nonlinear propagation.

An anthropomorphic breast phantom developed under grant funding has been used widely by other investigators and clinicians to calibrate their ultrasound imaging systems. A similar approach is also being applied to the development of phantoms for magnetic resonance systems.

Miscellaneous

There are several miscellaneous grants. One is a multi-modality study employing magnetic resonance and computed tomography studying the effects of therapy on brain tumors. Another employs magnetic resonance spectroscopy and positron emission tomography in the evaluation of tumor metabolism. One grant is involved with the biological effects of electron spin resonance, and another supports diaphanography, evaluating the specificity and sensitivity of this modality with respect to breast cancer. Still another grant supports the rapid reconstruction of images produced by computed tomography and magnetic resonance methodologies.

RRP/NCI PROGRAM SUPPORTED RESEARCH

1) COMPARATIVE CLINICAL MRI STUDIES
NCI Contract No. CM-47564-27

Five institutions are participating in this contract (a) to develop criteria and guidelines for clinical use of nuclear magnetic resonance imaging (MRI) in the non-invasive characterization of tissues and (b) to carry out performance and evaluation studies to compare the diagnostic accuracy of MRI with other modalities (e.g., CT and radioisotope imaging).

Accrual of cases is being satisfactorily achieved in 7 protocols: Brain neoplasms, lung neoplasms, musculo-skeletal tumors, uterine neoplasms, liver metastases, cervical myelopathies and congenital heart disease. Double blind review of case material has been initiated and computer entry of data begun.

2) BASIC RESEARCH FACTORS INFLUENCING NUCLEAR MAGNETIC RESONANCE RELAXATION TIMES IN BIOLOGICAL TISSUES
NCI RFA 84-CA-16

Fundamental, theoretical and experimental research is being carried out by four different projects which are attempting to understand the mechanisms responsible for the magnetic relaxation times T_1 and T_2, parameters which significantly determine image contrast in MRI and may one day permit the noninvasive characterization of normal and pathological tissues. Reports on this study are not yet available.

3) NATIONAL COLLABORATIVE DIAGNOSTIC IMAGING TRIALS
REQUEST FOR COOPERATIVE AGREEMENT (NCI 86-CA-10)

The availability of Request for Cooperative Agreement Applications was announced on May 23, 1986. The Request for Applications has an application receipt date of September 24, 1986. This research effort represents the first admission of the Diagnostic Imaging Research Branch to the arena of cooperative clinical trials and is most timely and appropriate. With the explosion of advanced technologies, it is fortunate to have the means of testing the efficacy of the various technologies in the locations for which they are intended - namely the clinical settings. Multi-institutional trials are needed to assess the relative role of each imaging modality in

cancer management. The specific objective of this RFA is to support multicenter cooperative clinical trials to determine the most effective imaging procedures required to stage and monitor carcinoma of the lung and prostate. The end result off this project will be to develop specific decision trees or algorithms for the appropriate sequential use of state-of-the-art imaging procedures applied to the diagnostic staging and timely follow-up of these specific tumors.

SMALL BUSINESS INNOVATIVE RESEARCH - GRANTS AND CONTRACTS

Application for these awards continues to be brisk and a number of interesting applications and proposals have been made. In many instances, the Small Business is affiliated with a University Center and, consequently, the quality of many applications is quite good.

Grants

During the past year, the Diagnostic Imaging Research Branch (DIRB) has supported seven Phase I grants and seven Phase II grants. The Phase I grants have dealt with ultrasound instrumentation, NMR contrast agents, thermography for home use, radio-immuno-detection and low cost medical imaging.

The Phase II grants have been involved with computed ultra-sonography, holographic ultra-sonography, radiopharmaceutical development and physical/engineering instrumentation.

Contracts

The contracts awarded in late FY85 have investigated diverse fields as physics, engineering instrumentation, radiopharmaceutical development, ultrasound display, magnetic resonance contrast agents, magnetic resonance spectroscopy, laser cancer detection and digital color image display.

WORKSHOPS

Two Workshop were conducted in this fiscal year.

"Cell Specific Diagnosis Employing Radiolabeled Agents"

This meeting discussed the subject of monoclonal antibodies and radio-labeled specific binding substances as they relate to imaging. Among the recommendations for future research that resulted from the Workshop were: improvement of the efficacy of the antibody labeling technique for imaging, reduction of immunogenicity of monoclonal antibodies, development of human hybrids or chimeras that may have less immunogenicity, the advantages/disadvantages of monoclonal vs. polyclonal antibodies for imaging, development of a valid animal model to screen antibodies for human use, a search for new antigens which may better develop antibodies, and study of the advantages/disadvantages of metal chelates vs. radioiodine in radioimmuno-imaging.

WORKSHOPS - (Continued)

"Tissue Characterization by Radiologic Imaging: Regional Metabolism and Tissue Perfusion"

This mini-workshop was concerned with the application of the more recently developed technologies (magnetic resonance imaging and spectroscopy, positron emmission tomography, digital radiography) toward improved accuracy of diagnosis in both the anatomical and functional aspects of disease with special emphasis on regional metabolism. Further inquiry using these methodologies is expected to reveal information concerning changes in metabolism such as might occur either with the natural history of the lesion or as a result of intervention, for example, with radiation therapy. The mini-workshop served as an agenda preparing meeting for a full-scale workshop on this same subject to be held in October 1986.

FUTURE PLANS

Objectives and goals for Diagnostic Imaging continue to center around sensitivity and specificity of diagnosis achieved with non-invasive technology. The following principal directions of investigation continue to occupy our attention:

1) Further improvement in the development of radiolabeled agents for detection and staging of cancer and monitoring of therapy.

2) The application of the more recently developed technologies (magnetic resonance) imaging and spectroscopy, positron emission tomography, digital radiography, computed tomography) toward improved accuracy of diagnosis in both the anatomical and functional aspects of disease. The objective is the depiction of metabolism related to specific anatomic sites by the employment of imaging and imaging related methods.

3) Basic research for improvement in digital radiography and ultrasonography, and development of new radiopharmaceuticals (for SPECT, PET) aimed at sensitivity and specificity of diagnosis, both anatomical and functional, occupy a high priority in the Diagnostic Imaging Research Branch.

FY86 Annual Report Summary
Radiotherapy Development Branch

The Radiotherapy Development Branch (RDB) administers a large program of basic, developmental, and clinical research related to cancer treatment modalities utilizing ionizing or nonionizing radiations and the investigation of means of modifying the biological effects of these radiations. This body of research covers a range of scientific disciplines including radiation biology, radiation chemistry, radiation physics, and radiation oncology. Research efforts range from the investigation of basic interaction mechanisms between radiation and biological systems to controlled clinical trials for a multitude of disease sites treated with single or multi-modality treatment schemas.

Areas of basic research supported by RDB have generated leads for promising new treatment modalities that are currently being tested in clinical trials. Among these are particle therapy, intraoperative radiotherapy, radiation modifiers, and photodynamic therapy. Radiation modifiers include radioprotective agents which reduce normal tissue morbidity, radiosensitizers which enhance the effects of radiation on tumors but not in normal tissues, and hyperthermia which also preferentially affects tumor tissues.

Major areas of research in terms of funding are particle radiotherapy, hyperthermia, and general radiobiology. Substantial support is also provided for the development of radiosensitizers and radioprotectors, intraoperative radiotherapy, photodynamic therapy, and radiation physics.

The largest dedicated portion of the Radiotherapy Development Branch budget supports particle radiotherapy. This program goes back over a decade and now funds six clinical research facilities: four neutrons, one proton, and one helium/heavier ion. The four neutron facilities include the University of Texas/M.D. Anderson, University of California at Los Angeles, the University of Washington and the Cleveland Clinic Foundation, which collaborate as a neutron clinical trials group with common protocols.

Three of the five neutron Phase II dose searching studies (thorax, head and neck, pelvis) have met their accrual objectives. Based on the results of these studies, 20 Gy in 12 fractions over 4 weeks will be used in subsequent studies. Phase III studies for head and neck and prostate have been activated. Phase III studies for cervix and lung should be opened by the end of FY86. A Phase III study for salivary glands has been completed and shows neutrons to be the treatment of choice.

At the Lawrence Berkeley Lab of the University of California research continues on the therapeutic applications of heavy charged particles such as helium, carbon, neon and silicon ions in the treatment of human cancers. Because of the precise dose delivery possible with these radiation beams, improved tumor control rates of 80-90% have been achieved in uveal melanoma and paraspinal-base of skull chordoma and chondrosarcoma. Mean follow up in these patient groups has now reached about 30 months. For selected tumors of the head and neck area such as paranasal sinuses or salivary glands, and for soft tissue tumors, local control rates of 60% have been obtained using both helium and heavier ions with median survival not yet

reached at 32 months. Beginning studies in locally advanced carcinoma of the prostate and unresectable carcinoma of the lung appear sufficiently promising for Phase III trials.

Research on the therapeutic potential of proton irradiation continues at the Harvard Cyclotron Lab. A total of 88 patients with chordomas or low grade chondrosarcomas of the base of the skull of the cervical spine have received proton radiation treatments. New patients continue to be referred at a rate of approximately 24 per year. The results of treatment for the first 63 patients have recently been analyzed. The median follow-up time is 27 months. The five year actuarial results for the 50 patients with base of skull tumors are 78% for local control and 76% for disease-free survival. Seven of the thirteen patients with cervical spine tumors have had no recurrence of disease. This continues to compare favorably with the 25 - 30% local control rate reported in the literature.

The most rapidly growing area of research support by the Radiotherapy Development Branch continues to be loco-regional hyperthermia in conjunction with radiation.

Interest in the use of hyperthermia as an adjunct to radiation and chemotherapy continues widespread in the radiation research community. Both the preclinical and clinical studies continue to proliferate. The mechanism by which hyperthermia effects cells is being studied in many different ways. One study is looking at the bulk electrical properties of tumor and normal tissues, at frequencies that are consistent with hyperthermic applications. The mechanism by which intracellular pH decreases during and after hyperthermia is being investigated using EMT6 multicellular spheroids. In vivo ^{31}P nuclear magnetic resonance spectroscopy has been used to monitor the response of murine tumors to hyperthermia. Dose related decreases in ATP and pH, with a simultaneous increase in phosphorus were observed. These changes were associated with a simultaneous and proportional decrease in tumor blood flow. The preliminary data suggests that the animals showing the greatest decrease in ATP showed the greatest cell kill as determined by a clonogenicity assay. Heat radiosensitization was shown in another study to correlate very well with the loss of activity of the repair enzyme, polymerase beta. There have been numerous studies investigating the mechanisms of thermotolerance. It has been shown that mild heating enhances the rate of synthesis and accumulation of heat shock proteins in murine tumors and normal tissues. The increased cellular concentrations of these proteins correlate well with transient thermotolerance or stable heat resistance in vitro. A more detailed study suggests that the thermotolerance (thermoresistance) associated with slow heating is not a result of changes in membrane cholesterol content, fatty acid content, cellular protein, or cell cycle distribution. It was also noted in this study that the heat shock protein 70, which is induced by immediate heating, was not synthesized at the same high rate by thermotolerant cells. However, the recent observation that cell membranes enriched with mono-unsaturated fatty acids have a decreased heat sensitivity lends support to the possibility that thermotolerant cells might acutally utilize the mechanism of alteration of membrane lipids to achieve their sensitive state. Thermotolerance has also been attributed to cellular glutathione which protects cells from the oxidative damage produced during hyperthermia treatment. The results of two separate studies have advanced the concept that intrinsic

heat sensitivity of cells depends upon the critical level of cellular energy at the time of heat exposure. The class of compounds known as energy depletors were found to be excellent heat sensitizers. Several studies have attempted to model the heat transfer properties of tissues, with an aim of improving the controllability and predictability of hyperthermia treatments. The models are expected to provide a better understanding of the mechanisms of heat transfer in tissues, in particular in the contribution of vessel geometry and equilibration lengths to the heat clearance by blood flow in tissue. As practical tools, the models closely approximate the bioheat equation and can be useful in testing and designing hyperthermia applicators for improved patient treatments. Mathematical models have been developed to supplement the limited temperature measurements obtained clinically to estimate complete temperature fields during hyperthermia. The models will also be used to determine how best to distribute power to produce uniform, therapeutic temperatures at all points inside a tumor. Evaluation of the models will be accomplished with phantoms and clinically. New dynamic phantoms are being developed for testing a variety of hyperthermia devices. The use of canine kidneys in vivo as thermal models for evaluating the effectiveness of different types of hyperthermia equipment and configurations has been proposed. Clinically, the evaluation of hyperthermia devices is an ongoing effort. New phased array microwave systems and scanned focused ultrasonic techniques have been developed for deep heating applications. Interstitial multiple microwave antenna arrays have been developed which can shape temperature distributions for controlled hyperthermia of non-superficial tumors. A number of microwave intracavitary probes have been developed and are being tested. One new method involves the implantation of an array of needle-shaped 'thermoseeds' into the tumor which become hot when the patient is inserted into a magnetic induction coil. The thermoseeds are made of a special alloy which provides for some automatic temperature regulation. A new concept in hyperthermia has also been the development of treatment planning systems. A system applied to ferromagnetic implants has been refined and is now available for use in humans. It has been used to evaluate heating of canine brains with ferromagnetic implants. The model is now being extended to three dimensions.

The results of Phase I clinical trials in hyperthermia indicate that improved tumor control following radiation or chemotherapy can be achieved with the concomitant use of heat. Phase II-III clinical trials have been initiated in localized superficial tumors (head and neck).

Basic research in the fields of radiosensitizers and radioprotectors is proceeding well.

Several new classes of potential non-nitro radiosensitizer compounds have been identified. Analogs of benzamide and nicotinamide, NSC 604583 and NSC 605533 respectively, have shown sufficient radiosensitizing activity and have been recommended as candidates for the NCI Decision Network. These compounds are being structurally modified to improve their solubility, lipophilicity and toxicity. A benzotriazine, SR-4233, has been shown to be selectively cytotoxic to hypoxic tumor cells. Because of this property, this compound could be an extremely effective anti-cancer drug. A halopyrimidine, 5-chlorodeoxyuridine, has also been shown to be effective as a radiosensitizer. Basic research is continuing to better define the mechanism of radiosensitization. The Phase I of the radiosensitizer SR-2508 has been

completed and the maximal starting dose for the projected Phase II-III trials determined. The dose-limiting toxicity was peripheral neuropathy. Pharmacokinetic data for an individual patient is predictive of the risk of the patient developing peripheral neuropathy and can be used to modify treatment to reduce this risk. Phase II-III trials of SR-2508 are being initiated in head and neck cancers and some pilot studies are projected for other anatomical sites. Phase I clinical trials of perfluorochemicals as radiosensitizers have been initiated using Fluosol plus an oxygen rich atmosphere.

Most of the research in radioprotection involves the mechanisms of radioprotection by aminothiol compounds, specifically WR-2721. Work in this area has been slowed by the problems of identifying and quantitating the metabolic products of WR-2721 in plasma and tissue. WR-2721 is rapidly degraded once administered in vivo into numerous sulfur containing compounds. An intensive effort was made to determine the pharmacokinetics of WR-2721 over the past few years and it is starting to show positive results. Investigators have sought to establish the mechanisms underlying the selective protection action of WR-2721 on normal tissue. Alkaline phosphatase, a cell surface enzyme which is found in high concentrations in capillaries, appears to play a key role in converting WR-2721, which is not taken up by cells, to WR-1065, which is taken up and whose intracellular levels correlate with radioprotection. Tumor cells appear to have less alkaline phosphatase activity and take up less WR-1065. The ability of thiols to protect DNA correlates with their ability to bind to DNA. Other studies have shown that WR-1065 is capable of protecting against radiation-induced mutations, even when administered three hours following irradiation. In vivo studies on mice have shown that WR-2721 is effective in treating lymphoma. The slight lymphoma radioprotection is more than counterbalanced by the cytotoxicity of WR-2721 against this tumor, with a resultant therapeutic gain of 2.9. WR-2721 and WR-77913 have been found effective in protecting rats against gamma radiation induced cataracts of the lens.

The Phase I clinical trial of a single dose of WR-2721 has been completed and 740 mg/M^2 was defined as the maximum tolerated dose. Pharmacokinetic studies are being done as part of this protocol. The multiple dose study of WR-2721 has also been completed with 340 mg/M^2 being the maximum tolerated dose that can be delivered four times a week over a period of five weeks. Additional pilot studies are exploring the efficacy of WR-2721 as a chemomodifier.

Preclinical research in photodynamic therapy (PDT) continues to be active. Many of the problem areas in instrumentation, light delivery systems, light dosimetry and photoactive chemicals are under investigation. Basic photochemical and photobiological actions of drugs (porphyrins) and light are being studied at the molecular, cellular and tissue levels. Phase I clinical trials have been completed and a maximal starting dose for drug and light with acceptable toxicity has been determined. Protocols have been developed for the Phase II-III clinical trials which will be initiated. The recent participation of a pharmaceutical company in making and supplying the drug (Photofrin II) now being used in PDT will provide an added stimulus to the conduct of clinical trials using PDT as a treatment modality.

Basic research into improved characterization of the physics of various radiotherapy modalities continues. The fifth of a planned five groups of contract supported studies was funded in FY86. These include: (1) treatment planning intercomparisons for heavy particles including neutrons, protons, helium and heavier ions; (2) treatment planning intercomparisons for external photon beams; (3) treatment planning intercomparisons for interstitial radiotherapy; (4) dose calculations for radiotherapy using labeled antibodies directed to tumor associated and/or tumor specific antigens; and (5) treatment planning intercomparisons for external electron beams. These five groups of contracts should lead to a comprehensive evaluation of computerized treatment planning and point the way to future advances.

The RDB grant program funds a variety of interesting and exciting research efforts relevant to clinical radiotherapy physics. Computer-controlled radiotherapy allows versatile field shaping. Beam configuration and patient anatomy are visualized in three dimensions with anatomy information derived from CT and/or MRI images. Studies are continuing for achieving prescribed dose distributions with various combinations of accelerator motions including the use of a multileaf collimator. As the complexity and automation of radiotherapy delivery increases, the need for treatment planning dosimetry verification becomes more acute. The NCI is supporting the American Association of Physicists in Medicine in developing a benchmark standard to provide users of treatment planning computer systems with the ability to understand and document computation accuracy limitations.

The NCI, primarily through the Radiotherapy Development Branch, continues to support a major portion of Radiation Biology research in the U.S. which is dedicated to improving radiation therapy as a treatment modality. Tumor and normal tissue radiobiology at the molecular, cellular and whole animal levels continues to be vigorously researched.

The following illustrates the breadth and diversity of this program. Research on the topics listed is being supported:

(1) The effects of radiation on inhibiting natural killer cell activity in humans (natural killer cells have been shown to play a role in the destruction of tumor cells)

(2) A study of the influence of dose per fraction on the heart and other mediastinal structures - it indicates an increased risk for late normal tissue complications with increasing size of dose per fraction

(3) A newly developed tissue culture assay which measures cell death of both dividing and non-dividing cell populations offers new insights into the mechanism and timing of radiation death (The standard colony assay system works only on dividing cell populations, while the majority of cells in living organisms do not continually undergo cell division)

(4) A study quantifying the amount of residual radiation damage in an acutely responding normal tissue (skin) and a late responding normal tissue (lung), as a function of initial dose and time between initial and retreatment dose (testing the hypothesis that the time and rate of decay of residual damage varies between acute and late responding normal tissues dependent on the kinetics of cell depletion specific to the tissue under test. The lung results do not fit this hypothesis but rather suggested that there is a component of lung damage expressed at early times, at least at higher doses.)

(5) The development of radiation sensitive mutants in mammalian cells for furthering the understanding of the relationships between the induction of DNA damage and cell killing (At the molecular level data on such cells suggest that intrinsic radiosensitively of mammalian cells is a reflection of their capacity for repair of double strand breaks.)

(6) Preliminary laboratory studies with radiation and drug combinations indicate that many drugs in common clinical use for tumor chemotherapy can increase the effectiveness of irradiation when administered at appropriate times (This suggests that improved treatment of human disease may be achievable simply by appropriate integration of radiotherapy and chemotherapy.)

(7) A study to measure changes in vascular permeability that occur in response to radiation exposure in order to relate them to long term effects such as radiation fibrosis (Development of fibrosis is often the dose limiting factor in radiotherapy.)

(8) A study to test the usefulness of high pressure oxygen and artificial blood (fluosol) to overcome the problem of radioresistent hypoxic cells in tumors (Animal studies suggest that the treatment is safe for use with radiotherapy and that a therapeutic gain can be obtained.)

(9) A study of the repair kinetics of a variety of normal tissues because of the potential use of hyperfractionation schemes to spare late-responding normal tissue in radiotherapy

(10) The development of techniques to culture and measure the intrinsic radiosensitivity of cells taken directly from human tumors (This assay is of potential significance in deciding between different therapeutic options for individual patients.)

The RDB relies heavily for guidance on new research initiatives on advice and counsel of the radiation research community expecially as enumerated by the Interdisciplinary Program for Radiation Oncology Research, developed with NCI support, by a group of leading scientists under the aegis of the American College of Radiology, Commission on Cancer. An update of this plan is underway under the same auspices for publication in 1987.

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